

NOVEL PHOSPHORUS-CONTAINING PRODRUGS

Related Application

This application is a continuation-in-part of Provisional Application Serial No.
5 60/153,127, filed September 8, 1999, and Provisional Application Serial No. 60/123,013,
filed March 5, 1999, and they are incorporated by reference in their entirety.

Field of the Invention

The present invention is directed towards novel prodrugs that generate
10 phosph(on)ate compounds which are biologically active or are further phosphorylated to
produce biologically active compounds, to their preparation, to their synthetic
intermediates, and to their uses. More specifically, the invention relates to the area of
substituted cyclic 1,3-propanyl esters wherein the cyclic moiety contains at least one
amino attached to the phosphorus.

Background of the Invention

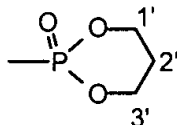
The following description of the background of the invention is provided to aid in
understanding the invention, but is not admitted to be, or to describe, prior art to the
invention. All cited publications are incorporated by reference in their entirety.

20 Free phosphorus and phosphonic acids and their salts are highly charged at
physiological pH and therefore frequently exhibit poor oral bioavailability, poor cell
penetration and limited tissue distribution (*e.g.* CNS). In addition, these acids are also
commonly associated with several other properties that hinder their use as drugs, including
short plasma half-life due to rapid renal clearance, as well as toxicities (*e.g.* renal,
25 gastrointestinal, *etc.*) (*e.g.* *Antimicrob Agents Chemother* 1998 May; 42(5): 1146-50).
Phosphates have an additional limitation in that they are not stable in plasma as well as
most tissues since they undergo rapid hydrolysis via the action of phosphatases (*e.g.*
alkaline phosphatase, nucleotidases).

Prodrugs of phosphorus-containing compounds have been sought primarily to
30 improve the limited oral absorption and poor cell penetration. In contrast to carboxylic
acid proesters, many phosphonate and phosphate esters fail to hydrolyze *in vivo*, including
simple alkyl esters. The most commonly used prodrug class is the acyloxyalkyl ester,

which was first applied to phosphate and phosphonate compounds in 1983 by Farquhar *et al.*, *J. Pharm. Sci.* 72(3):324 (1983).

Cyclic phosphonate and phosphate esters have also been described for phosphorus-containing compounds. In some cases, these compounds have been investigated as potential phosphate or phosphonate prodrugs. Hunston *et al.*, *J. Med. Chem.* 27: 440-444 (1984). The numbering for these cyclic esters is shown below:



- 10 The cyclic 2',2'-difluoro-1',3'-propane ester is reported to be hydrolytically unstable with rapid generation of the ring-opened monoester. Starrett *et al.* *J. Med. Chem.* 37: 1857-1864 (1994).

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Cyclic 3',5'-phosphate esters of araA, araC and thioinosine have been synthesized. Meier *et al.*, *J. Med. Chem.* 22: 811-815 (1979). These compounds are ring-opened through the action of phosphodiesterases which usually require one negative charge.

Cyclic 1',3'-propanyl phosphonate and phosphate esters are reported containing a fused aryl ring, i.e. the cyclosaligenyl ester, Meier *et al.*, *Bioorg. Med. Chem. Lett.* 7: 99-104 (1997). These prodrugs are reported to generate the phosphate by a "controlled, non-enzymatic mechanism[s] at physiological pH according to the designed tandem-reaction in two coupled steps". The strategy was purportedly used to deliver d4-T monophosphate to CEM cells and CEM cells deficient in thymidine kinase infected with HIV-1 and HIV-2.

Unsubstituted cyclic 1',3'-propanyl esters of the monophosphates of 5-fluoro-2'-deoxy-uridine (Farquhar *et al.*, *J. Med. Chem.* 26: 1153 (1983)) and ara-A (Farquhar *et al.*, *J. Med. Chem.* 28: 1358 (1985)) were prepared but showed no *in vivo* activity. In addition, cyclic 1',3'-propanyl esters substituted with a pivaloyloxy methyloxy group at C-1' was prepared for 5-fluoro-2'-deoxy-uridine monophosphate (5-FdUMP; (Freed *et al.*, *Biochem. Pharmac.* 38: 3193 (1989); and postulated as potentially useful prodrugs by others (Biller *et al.*, US 5,157,027). In cells, the acyl group of these prodrugs underwent cleavage by esterases to generate an unstable hydroxyl intermediate which rapidly broke down to the free phosphate and acrolein following a β -elimination reaction as well as formaldehyde and pivalic acid.

Unsubstituted cyclic phosphoramidate esters, i.e. cyclic phosphonate and phosphate esters wherein one of the ring oxygens is replaced with an NR, are also known. For example, cyclophosphamide (CPA) is representative of a class of mustard oncolytics that utilize this prodrug moiety. Although CPA is activated primarily in the liver via a cytochrome P450-catalyzed oxidation, its biological activity is outside the liver. CPA is an effective immunosuppressive agent as well as an oncolytic agent for extrahepatic cancers because one or more of the intermediate metabolites produced following P450 activation diffuses out of the liver and into the circulation. With time the intermediate(s) enter extrahepatic tissues and are thought to undergo a β -elimination reaction to generate acrolein and the active phosphoramidate mustard. Both products are reported to be cytotoxic to cells. The mustard cytotoxicity results from alkylation of DNA (or RNA). Acrolein is reported to exert its toxicity via several mechanisms, including depletion of glutathione, alkylation of DNA and proteins via a Michael reaction. In addition, acrolein produces other toxicities such as the dose-limiting bladder toxicity commonly observed with cyclophosphamide therapy. Since the toxicity of these agents often hampers their use as chemotherapy agents, numerous strategies are under investigation that are designed to enhance P450 activity in or near tumors and thereby localize the activation and antiproliferative effect of these agents to the tumor. One strategy uses retroviruses or other well known techniques for introducing genes into target tissues (e.g. Jounaidi et al., Cancer Research 58, 4391 (1998)). Other strategies include the placement of encapsulated cells engineered to produce cytochrome P450s (e.g. Lohr et al., Gene Therapy 5, 1070 (1998)) at or near the tumor.

Unsubstituted cyclic phosphoramidate esters have also been prepared as potential prodrugs of the nucleosides araA and 5-fluoro-2'-deoxyuridine (Farquhar et al., J. Med. Chem. 28, 1358-1361 (1985); J. Med. Chem. 26, 1153-1158 (1983)). The compounds were studied in a mouse model of leukemia where it was assumed that if the prodrug transformation was similar to cyclophosphamide, then these agents would be useful for treating a variety of cancers including leukemias as well as carcinomas of the colon, breast and ovary. In addition, since some of the mechanisms that account for tumor cell drug resistance entail a decrease in the enzymes used to synthesize the monophosphate, the strategy was expected to possibly be beneficial in treating drug resistant tumors. The compounds were only "marginally effective" in prolonging life span in the mouse model.

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A variety of substituted 1',3' propanyl cyclic phosphoramidates, wherein 1' represents the carbon alpha to the nitrogen were prepared as cyclophosphamide analogs (Zon, Progress in Med. Chem. 19, 1205 (1982)). For example, a number of 2'- and 3'-substituted proesters were prepared in order to decrease the propensity of the α,β -unsubstituted carbonyl by-product to undergo a Michael reaction. 2'-Substituents included methyl, dimethyl, bromo, trifluoromethyl, chloro, hydroxy, and methoxy whereas a variety of groups were used at the 3'-position including phenyl, methyl, trifluoromethyl, ethyl, propyl, i-propyl, and cyclohexyl. Analogs with a 3'-aryl group *e.g.* trans-4-phenylcyclophosphamide were "moderately effective in L1210 test system and showed no activity *in vivo*" G. Zu Prog. Med. Chem. 19: 205-246 (1982). A variety of 1'-substituted analogs were also prepared. In general these compounds were designed to be "pre-activated" cyclophosphamide analogs that bypass the oxidation step by already existing as a 1'-substituted analog capable of producing the final compound, *e.g.* hydroperoxide and 1-thioether. A series of 1'-aryl analogs were also prepared in order to enhance the oxidation potential. In contrast to the 1'-hydroperoxy analogs, the 1'-aryl compounds exhibited either no activity or very poor activity in the standard anticancer *in vivo* screen assay, *i.e.* the mouse L1210 assay. The lack of activity was postulated to arise from the steric hindrance of the phenyl and therefore limited oxidation of the prodrug. Support for this postulate was the potent activity of the acyclic phenyl keto analog which exhibited activity similar to cyclophosphamide. Luderman et al. J. Med. Chem. 29: 716 (1986).

Cyclic esters of phosphorus-containing compounds are reported in the chemical literature, however they were not tested as prodrugs in biological systems. These cyclic esters include:

[1] di and tri esters of phosphoric acids as reported in Nifantsev *et al.*, Phosphorus, Sulfur Silicon and Related Elements, 113: 1 (1996); Wijnberg *et al.*, EP-180276 A1;

[2] phosphorus (III) acid esters. Kryuchkov *et al.*, *Izv. Akad. Nauk SSSR, Ser. Khim.* 6: 1244 (1987). Some of the compounds were claimed to be useful for the asymmetric synthesis of L-Dopa precursors. Sylvain *et al.*, DE3512781 A1;

[3] phosphoramidates. Shih *et al.*, *Bull. Inst. Chem. Acad. Sin*, 41: 9 (1994); Edmundson *et al.*, *J. Chem. Res. Synop.* 5: 122 (1989); and

[4] phosphonates. Neidlein *et al.*, *Heterocycles* 35: 1185 (1993).

5 Numerous phosphorus-containing compounds are known to exhibit pharmacological activity but remain far from optimal due to one or more of the above-described limitations. Some of the activities described include phosphonic acids that are useful as antihypertensives and therapy for heart failure via inhibition of NEP 24.11, phosphonic acids that are useful for treating a variety of CNS conditions (stroke, epilepsy, 10 brain and spinal cord trauma, *etc.*) via binding to excitatory amino acid receptors (*e.g.* NMDA receptor), bisphosphonic acids that are useful for treating osteoporosis, phosphonic acids that are useful as lipid lowering agents (*e.g.* squalene synthase inhibitors), phosphonates that are useful in treating inflammation (*e.g.* collagenase inhibitors), phosphonates and phosphates that are useful in treating diabetes, cancer and 15 parasitic and viral infections.

Phosphates and phosphonates that are known to be particularly useful in glucose lowering activity and therefore are anticipated to be useful in treating diabetes are compounds that bind to the AMP site of fructose 1,6-bisphosphatase (FBPase) as described in US 5,658,889, WO 98/39344, WO 98/39343, and WO 98/39342. Other examples of phosphorus-containing drugs include squalene synthetase inhibitor (*e.g.* BMS 188494).

A large class of drugs known to be active against hepatitis are generally nucleoside or nucleotide analogs that are phosphorylated inside cells to produce the biologically active triphosphate. Examples include Lamivudine (3TC) and Vidarabine (araA). In each case, the drug interferes with viral replication via the triphosphate form through either inhibition of the viral DNA polymerases or DNA chain termination. Some specificity for virus-infected cells is gained by both preferential phosphorylation of the drug by virally-encoded kinases as well as by specific inhibition of viral DNA polymerases. Nevertheless, many of the nucleoside-based drugs are associated with significant non-hepatic toxicity. For example, araA frequently produces neurological toxicity (40%) with many patients showing myalgia or a sensory neuropathy with distressing pain and abnormalities in nerve conduction and a few showing tremor, dysarthria, confusion or even coma. Lok *et al.*, *J. Antimicrob. Chemotherap.* 14: 93-99 (1984). In other cases, the efficacy and/or

therapeutic index of nucleosides is compromised by poor phosphorylation efficiencies and therefore low levels of the biologically active triphosphate (e.g. Yamanaka et al., Antimicrob. Agents and Chemother. 43, 190 (1999)).

Phosphonic acids also show antiviral activity. In some cases the compounds are
5 antivirals themselves (e.g. phosphonoformic acid), whereas in other cases they require
phosphorylation to the diphosphate, e.g. 9-(2-phosphonylmethoxyethyl)adenine (PMEA,
Adefovir). Frequently, these compounds are reported to exhibit enhanced activity due to
either poor substrate activity of the corresponding nucleoside with viral kinases or because
the viral nucleoside kinase which is required to convert the nucleoside to the
10 monophosphate is down regulated viral resistance. Monophosphates and phosphonic
acids, however, are difficult to deliver to virally-infected cells after oral administration due
to their high charge and in the case of the monophosphate instability in plasma. In
addition, these compounds often have short half-lives (e.g. PMEA, Adefovir) due in most
cases to high renal clearance. In some cases, the high renal clearance can lead to
15 nephrotoxicities or be a major limitation in diseases such as diabetes where renal function
is often compromised.

Liver cancer is poorly treated with current therapies. In general, liver tumors are
resistant to radiotherapy, respond poorly to chemotherapy and are characterized by a high
degree of cell heterogeneity. Oncolytic nucleosides such as 5-fluoro-2'-deoxyuridine,
20 have also shown a poor response against primary liver cancers.

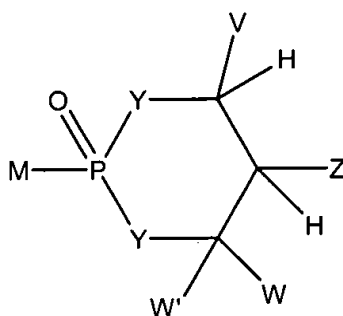
Summary of the Invention

The present invention is directed towards novel prodrugs that generate
phosph(on)ate compounds, their preparation, their synthetic intermediates, and their uses.
25 In one aspect, the invention is directed towards the use of the prodrugs to enhance oral
drug delivery. Another aspect of the invention is directed to the use of the prodrugs to
enhance the level of the biologically active drug in the liver. Another aspect of the
invention is the use of the prodrugs to treat diseases that benefit from enhanced drug
distribution or specificity to the liver and like tissues and cells, including hepatitis, cancer,
30 liver fibrosis, malaria, other viral and parasitic infections, and metabolic diseases where
the liver is responsible for the overproduction of the biochemical end product, e.g. glucose
(diabetes); cholesterol, fatty acids and triglycerides (hyperlipidemia) (atherosclerosis)
(obesity). In another aspect, the prodrugs are used to prolong pharmacodynamic half-life

of the drug. In addition, the prodrug methodology of the current invention is used to achieve sustained delivery of the parent drug. In another aspect, the prodrugs are used to increase the therapeutic index of the drug. Another aspect of the invention is the use of the prodrugs in combination with techniques that elevate P450 activity in specific tissues.

- 5 In another aspect of the invention, a method of making these prodrugs is described. A further aspect is the novel intermediates to these prodrugs. In another aspect, the prodrugs are also useful in the delivery of diagnostic imaging agents to the liver.

One aspect of the present invention concerns compounds that are converted *in vitro* or *in vivo* to the corresponding $M-PO_3^{2-}$, $MP_2O_6^{3-}$, $MP_3O_9^{4-}$, and $MP(O)(NHR^6)O^-$ and are
10 of formula I



I

wherein:

- V, W, and W' are independently selected from the group consisting of -H, alkyl,
15 aralkyl, alicyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, and 1-alkynyl; or

- together V and Z are connected via an additional 3-5 atoms to form a cyclic group containing 5-7 ring atoms, optionally 1 heteroatom, substituted with hydroxy, acyloxy, alkoxy, alkoxy, alkoxy, or aryloxy, attached to a carbon atom that is three atoms
20 from both Y groups attached to the phosphorus; or

together V and Z are connected via an additional 3-5 atoms to form a cyclic group, optionally containing 1 heteroatom, said cyclic group is fused to an aryl group at the beta and gamma position to the Y adjacent to V;

- together V and W are connected via an additional 3 carbon atoms to form an
25 optionally substituted cyclic group containing 6 carbon atoms and substituted with one substituent selected from the group consisting of hydroxy, acyloxy, alkoxy, alkoxy, alkoxy,

alkylthiocarbonyloxy, and aryloxycarbonyloxy, attached to one of said additional carbon atoms that is three atoms from a Y attached to the phosphorus;

together Z and W are connected via an additional 3-5 atoms to form a cyclic group, optionally containing one heteroatom, and V must be aryl, substituted aryl, heteroaryl, or substituted heteroaryl;

together W and W' are connected via an additional 2-5 atoms to form a cyclic group, optionally containing 0-2 heteroatoms, and V must be aryl, substituted aryl, heteroaryl, or substituted heteroaryl;

Z is selected from the group consisting of $-\text{CHR}^2\text{OH}$, $-\text{CHR}^2\text{OC}(\text{O})\text{R}^3$, $-\text{CHR}^2\text{OC}(\text{S})\text{R}^3$, $-\text{CHR}^2\text{OC}(\text{S})\text{OR}^3$, $-\text{CHR}^2\text{OC}(\text{O})\text{SR}^3$, $-\text{CHR}^2\text{OCO}_2\text{R}^3$, $-\text{OR}^2$, $-\text{SR}^2$, $-\text{CHR}^2\text{N}_3$, $-\text{CH}_2\text{aryl}$, $-\text{CH}(\text{aryl})\text{OH}$, $-\text{CH}(\text{CH}=\text{CR}^2_2)\text{OH}$, $-\text{CH}(\text{C}\equiv\text{CR}^2)\text{OH}$, $-\text{R}^2$, $-\text{NR}^2_2$, $-\text{OCOR}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{SCOR}^3$, $-\text{SCO}_2\text{R}^3$, $-\text{NHCOR}^2$, $-\text{NHCO}_2\text{R}^3$, $-\text{CH}_2\text{NHaryl}$, $-(\text{CH}_2)_p\text{-OR}^{12}$, and $-(\text{CH}_2)_p\text{-SR}^{12}$;

p is an integer 2 or 3;

with the provisos that:

a) V, Z, W, W' are not all -H; and

b) when Z is $-\text{R}^2$, then at least one of V, W, and W' is not -H, alkyl, aralkyl, or alicyclic;

R^2 is selected from the group consisting of R^3 and -H;

R^3 is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl;

R^6 is selected from the group consisting of -H, lower alkyl, acyloxyalkyl, alkoxycarbonyloxyalkyl, and lower acyl;

R^{12} is selected from the group consisting of -H, and lower acyl;

each Y is independently selected from the group consisting of $-\text{O}-$, $-\text{NR}^6-$ with the proviso that at least one Y is $-\text{NR}^6-$;

M is selected from the group that attached to PO_3^{2-} , $\text{P}_2\text{O}_6^{3-}$, $\text{P}_3\text{O}_9^{4-}$, or

$\text{P}(\text{O})(\text{NHR}^6)\text{O}^-$ is a biologically active agent, but is not an FBPase inhibitor, and is attached to the phosphorus in formula I via a carbon, oxygen, sulfur or nitrogen atom;

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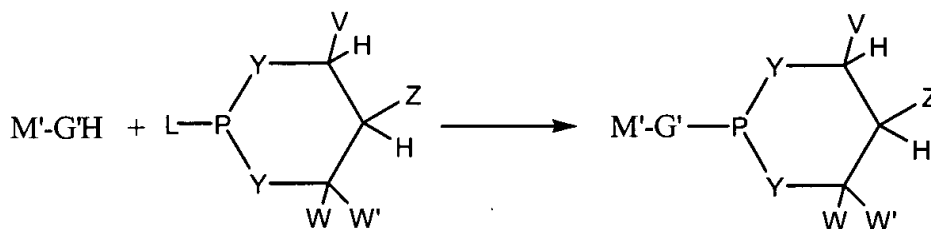
with the provisos that:

1) M is not $\text{-NH}(\text{lower alkyl})$, $\text{-N}(\text{lower alkyl})_2$, $\text{-NH}(\text{lower alkylhalide})$, $\text{-N}(\text{lower alkylhalide})_2$, or $\text{-N}(\text{lower alkyl})(\text{lower alkylhalide})$; and

2) R^6 is not lower alkylhalide;

5 and pharmaceutically acceptable prodrugs and salts thereof.

The present invention provides several novel methods of making the prodrugs of the present invention. One method relies on the reaction of the following novel P(III) reagent:

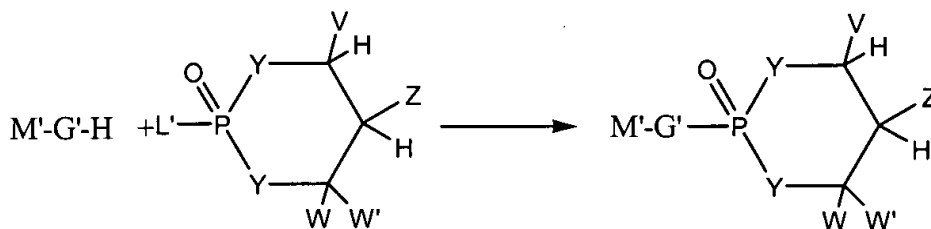


$\text{G}'=\text{O}, \text{NR}$

$\text{L}=\text{leaving groups such as } \text{-NR}_2, \text{halogen}$

10 The resulting phosphite is then oxidized to the cyclic phosphoramidate.

A second method relies on the reaction of a novel P(V) reagent:

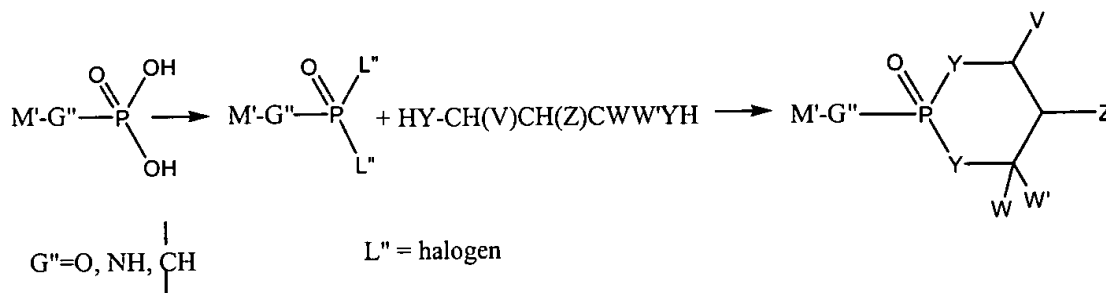


$\text{G}'=\text{O}, \text{NH}$

$\text{L}'=\text{leaving groups such as } \text{-NR}_2, \text{-O-aryl, halogen}$

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A third method relies on reacting another novel P(V) compound with a diamine or amino alcohol:



Since these compounds have asymmetric centers, the present invention is directed not only to racemic and diastereomeric mixtures of these compounds, but also to individual stereoisomers. The present invention also includes pharmaceutically acceptable and/or useful salts of the compounds of formula I, including acid addition salts. The present inventions also encompass prodrugs of compounds of formula I.

Definitions

In accordance with the present invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise.

The term "aryl" refers to aromatic groups which have 5-14 ring atoms and at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. Suitable aryl groups include phenyl and furan-2,5-diyl.

Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups and polycyclic or fused compounds such as optionally substituted naphthyl groups.

Heterocyclic aryl or heteroaryl groups are groups having from 1 to 4 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms being carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen. Suitable heteroaryl groups include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolyl, pyridyl-N-oxide, pyrimidyl, pyrazinyl, imidazolyl, and the like, all optionally substituted.

The term "biaryl" represents aryl groups containing more than one aromatic ring including both fused ring systems and aryl groups substituted with other aryl groups. Such

groups may be optionally substituted. Suitable biaryl groups include naphthyl and biphenyl.

The term "alicyclic" means compounds which combine the properties of aliphatic and cyclic compounds. Such cyclic compounds include but are not limited to, aromatic, cycloalkyl and bridged cycloalkyl compounds. The cyclic compound includes heterocycles. Cyclohexenylethyl and cyclohexylethyl are suitable alicyclic groups. Such groups may be optionally substituted.

The term "optionally substituted" or "substituted" includes groups substituted by one to four substituents, independently selected from lower alkyl, lower aryl, lower aralkyl, lower alicyclic, hydroxy, lower alkoxy, lower aryloxy, perhaloalkoxy, aralkoxy, heteroaryl, heteroaryloxy, heteroarylalkyl, heteroaralkoxy, azido, amino, guanidino, amidino, halo, lower alkylthio, oxo, acylalkyl, carboxy esters, carboxyl, -carboxamido, nitro, acyloxy, aminoalkyl, alkylaminoaryl, alkylaryl, alkylaminoalkyl, alkoxyaryl, arylamino, aralkylamino, phosphono, sulfonyl, -carboxamidoalkylaryl, -carboxamidoaryl, hydroxyalkyl, haloalkyl, alkylaminoalkylcarboxy-, aminocarboxamidoalkyl-, cyano, lower alkoxyalkyl, lower perhaloalkyl, and arylalkyloxyalkyl. "Substituted aryl" and "substituted heteroaryl" preferably refers to aryl and heteroaryl groups substituted with 1-3 substituents. Preferably these substituents are selected from the group consisting of lower alkyl, lower alkoxy, lower perhaloalkyl, halo, hydroxy, and amino.

The term "-aralkyl" refers to an alkylene group substituted with an aryl group. Suitable aralkyl groups include benzyl, picolyl, and the like, and may be optionally substituted. "Heteroarylalkyl" refers to an alkylene group substituted with a heteroaryl group.

The term "-alkylaryl" refers to an aryl group substituted with an alkyl group. "Lower -alkylaryl" refers to such groups where alkyl is lower alkyl.

The term "lower" referred to herein in connection with organic radicals or compounds respectively defines such as with up to and including 10, preferably up to and including 6, and advantageously one to four carbon atoms. Such groups may be straight chain, branched, or cyclic.

The terms "arylamino" (a), and "aralkylamino" (b), respectively, refer to the group -NRR' wherein respectively, (a) R is aryl and R' is hydrogen, alkyl, aralkyl or aryl, and (b) R is aralkyl and R' is hydrogen or aralkyl, aryl, alkyl.

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The term "acyl" refers to $-C(O)R$ where R is alkyl and aryl.

The term "carboxy esters" refers to $-C(O)OR$ where R is alkyl, aryl, aralkyl, and alicyclic, all optionally substituted.

The term "carboxyl" refers to $-C(O)OH$.

5 The term "oxo" refers to $=O$ in an alkyl group.

The term "amino" refers to $-NRR'$ where R and R' are independently selected from hydrogen, alkyl, aryl, aralkyl and alicyclic, all except H are optionally substituted; and R and R¹ can form a cyclic ring system.

10 The term "-carboxylamido" refers to $-CONR_2$ where each R is independently hydrogen or alkyl.

The term "halogen" or "halo" refers to $-F$, $-Cl$, $-Br$ and $-I$.

The term "alkylaminoalkylcarboxy" refers to the group $alkyl-NR-alk-C(O)-O-$ where "alk" is an alkylene group, and R is a H or lower alkyl.

15 The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched chain and cyclic groups. Alkyl groups may be optionally substituted. Suitable alkyl groups include methyl, isopropyl, and cyclopropyl.

The term "cyclic alkyl" or "cycloalkyl" refers to alkyl groups that are cyclic of 3 to 10 atoms, more preferably 3 to 6 atoms. Suitable cyclic groups include norbornyl and cyclopropyl. Such groups may be substituted.

20 The term "heterocyclic" and "heterocyclic alkyl" refer to cyclic groups of 3 to 10 atoms, more preferably 3 to 6 atoms, containing at least one heteroatom, preferably 1 to 3 heteroatoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen. Heterocyclic groups may be attached through a nitrogen or through a carbon atom in the ring. The heterocyclic alkyl groups include unsaturated cyclic, fused cyclic and spirocyclic groups.
25 Suitable heterocyclic groups include pyrrolidinyl, morpholino, morpholinoethyl, and pyridyl.

The term "phosphono" refers to $-PO_3R_2$, where R is selected from the group consisting of $-H$, alkyl, aryl, aralkyl, and alicyclic.

30 The term "sulphonyl" or "sulfonyl" refers to $-SO_3R$, where R is H, alkyl, aryl, aralkyl, and alicyclic.

The term "alkenyl" refers to unsaturated groups which contain at least one carbon-carbon double bond and includes straight-chain, branched-chain and cyclic groups.

Alkenyl groups may be optionally substituted. Suitable alkenyl groups include allyl. "1-

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alkenyl” refers to alkenyl groups where the double bond is between the first and second carbon atom. If the 1-alkenyl group is attached to another group, *e.g.* it is a W substituent attached to the cyclic phosphoramidate, it is attached at the first carbon.

5 The term "alkynyl" refers to unsaturated groups which contain at least one carbon-carbon triple bond and includes straight-chain, branched-chain and cyclic groups. Alkynyl groups may be optionally substituted. Suitable alkynyl groups include ethynyl. "1-alkynyl" refers to alkynyl groups where the triple bond is between the first and second carbon atom. If the 1-alkynyl group is attached to another group, *e.g.* it is a W substituent attached to the cyclic phosphoramidate, it is attached at the first carbon.

10 The term "alkylene" refers to a divalent straight chain, branched chain or cyclic saturated aliphatic group.

The term "acyloxy" refers to the ester group $-O-C(O)R$, where R is H, alkyl, alkenyl, alkynyl, aryl, aralkyl, or alicyclic.

15 The term "aminoalkyl-" refers to the group NR_2 -alk- wherein "alk" is an alkylene group and R is selected from H, alkyl, aryl, aralkyl, and alicyclic.

The term "alkylaminoalkyl-" refers to the group alkyl-NR-alk- wherein each "alk" is an independently selected alkylene, and R is H or lower alkyl. "Lower alkylaminoalkyl-" refers to groups where each alkylene group is lower alkylene.

20 The term "arylaminoalkyl-" refers to the group aryl-NR-alk- wherein "alk" is an alkylene group and R is H, alkyl, aryl, aralkyl, and alicyclic. In "lower arylaminoalkyl-", the alkylene group is lower alkylene.

25 The term "alkylaminoaryl-" refers to the group alkyl-NR-aryl- wherein "aryl" is a divalent group and R is H, alkyl, aralkyl, and alicyclic. In "lower alkylaminoaryl-", the alkylene group is lower alkyl.

The term "alkoxyaryl-" refers to an aryl group substituted with an alkoxy group. In "lower alkoxyaryl-", the alkyl group is lower alkyl.

The term "aryloxyalkyl-" refers to an alkyl group substituted with an aryloxy group.

30 The term "aralkyloxyalkyl-" refers to the group aryl-alk-O-alk- wherein "alk" is an alkylene group. "Lower aralkyloxyalkyl-" refers to such groups where the alkylene groups are lower alkylene.

The term "alkoxy-" or "alkyloxy-" refers to the group alkyl-O-.

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The term "alkoxyalkyl-" or "alkyloxyalkyl-" refer to the group alkyl-O-alk- wherein "alk" is an alkylene group. In "lower alkoxyalkyl-", each alkyl and alkylene is lower alkylene.

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The terms "alkylthio-" and "alkylthio-" refer to the groups alkyl-S-.

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The term "alkylthioalkyl-" refers to the group alkyl-S-alk- wherein "alk" is an alkylene group. In "lower alkylthioalkyl-" each alkyl and alkylene is lower alkylene.

The term "alkoxycarbonyloxy-" refers to alkyl-O-C(O)-O-.

The term "aryloxy carbonyloxy-" refers to aryl-O-C(O)-O-.

The term "alkylthiocarbonyloxy-" refers to alkyl-S-C(O)-O-.

10

The terms "amido" or "carboxamido" refer to $\text{NR}_2\text{-C(O)-}$ and $\text{RC(O)-NR}^1\text{-}$, where R and R^1 include H, alkyl, aryl, aralkyl, and alicyclic. The term does not include urea, -NR-C(O)-NR- .

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The term "carboxamidoalkylaryl" and "carboxamidoaryl" refers to an aryl-alk- $\text{NR}^1\text{-C(O)}$, and $\text{ar-NR}^1\text{-C(O)-alk-}$, respectively where "ar" is aryl, "alk" is alkylene, R^1 and R include H, alkyl, aryl, aralkyl, and alicyclic.

The term "hydroxyalkyl" refers to an alkyl group substituted with one -OH.

The term "haloalkyl" and "alkylhalide" refers to an alkyl group substituted with one halo. Preferably, the halo is in the 2-position.

20

The term "cyano" refers to $\text{-C}\equiv\text{N}$.

The term "nitro" refers to -NO_2 .

The term "acylalkyl" refers to an alkyl-C(O)-alk-, where "alk" is alkylene.

The term "aminocarboxamidoalkyl-" refers to the group $\text{NR}_2\text{-C(O)-N(R)-alk-}$ wherein R is an alkyl group or H and "alk" is an alkylene group. "Lower aminocarboxamidoalkyl-" refers to such groups wherein "alk" is lower alkylene.

25

The term "heteroarylalkyl" refers to an alkyl group substituted with a heteroaryl group.

The term "perhalo" refers to groups wherein every C-H bond has been replaced with a C-halo bond on an aliphatic or aryl group. Suitable perhaloalkyl groups include -CF_3 and -CFCI_2 .

30

The term "guanidino" refers to both -NR-C(NR)-NR_2 as well as $\text{-N=C(NR}_2)_2$ where each R group is independently selected from the group of -H, alkyl, alkenyl, alkynyl, aryl, and alicyclic, all except -H are optionally substituted.

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The term "amidino" refers to $-C(NR)-NR_2$ where each R group is independently selected from the group of -H, alkyl, alkenyl, alkynyl, aryl, and alicyclic, all except -H are optionally substituted.

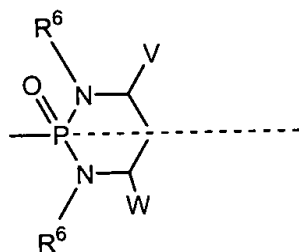
5 The term "pharmaceutically acceptable salt" includes salts of compounds of formula I and its prodrugs derived from the combination of a compound of this invention and an organic or inorganic acid or base. Suitable acids include HCl.

10 The term "prodrug" as used herein refers to any compound that when administered to a biological system generates a biologically active compound as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination of each. Standard prodrugs are formed using groups attached to functionality, *e.g.* HO-, HS-, HOOC-, R_2N -, associated with the drug, that cleave *in vivo*. Standard prodrugs include but are not limited to carboxylate esters where the group is alkyl, aryl, aralkyl, acyloxyalkyl, alkoxy-carbonyloxyalkyl as well as esters of hydroxyl, thiol and amines where the group attached is an acyl group, an alkoxy-carbonyl, 15 aminocarbonyl, phosphate or sulfate. The groups illustrated are exemplary, not exhaustive, and one skilled in the art could prepare other known varieties of prodrugs. Such prodrugs of the compounds of formula I, fall within the scope of the present invention. Prodrugs must undergo some form of a chemical transformation to produce the compound that is biologically active or is a precursor of the biologically active compound. 20 In some cases, the prodrug is biologically active, usually less than the drug itself, and serves to improve drug efficacy or safety through improved oral bioavailability, pharmacodynamic half-life, etc. The biologically active compounds include, for example, anticancer agents, antiviral agents, and antibiotic agents.

25 The term "bidentate" refers to an alkyl group that is attached by its terminal ends to the same atom to form a cyclic group. For example, propylene imine contains a bidentate propylene group.

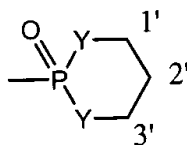
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The structure



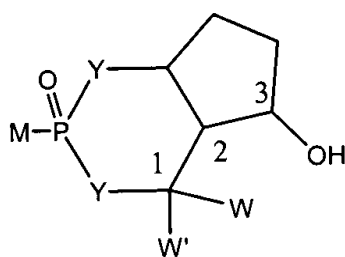
has a plane of symmetry running through the phosphorus-oxygen double bond when $R^6=R^6$, $V=W$, $W'=H$, and V and W are either both pointing up or both pointing down.

- 5 The term “cyclic 1',3'-propane ester”, “cyclic 1,3-propane ester”, “cyclic 1',3'-propanyl ester”, and “cyclic 1,3-propanyl ester” refers to the following:

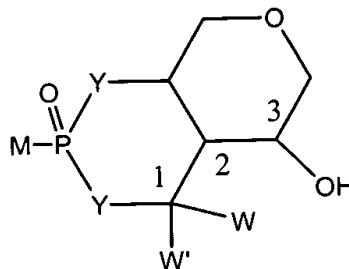


- 10 The phrase “together V and Z are connected via an additional 3-5 atoms to form a cyclic group containing 5-7 ring atoms, optionally containing 1 heteroatom, substituted with hydroxy, acyloxy, alkoxy, carbonyloxy, or aryloxy, carbonyloxy attached to a carbon atom that is three atoms from both Y groups adjacent to V ” includes the following:

The structure shown above (left) has an additional 3 carbon atoms that forms a five member cyclic group. Such cyclic groups must possess the listed substitution to be oxidized.



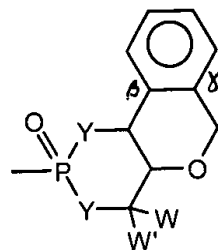
and



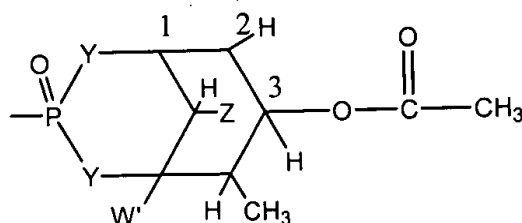
15

The phrase “together V and Z are connected via an additional 3-5 atoms to form a cyclic group, optionally containing one heteroatom, said cyclic group is fused to an aryl group attached at the beta and gamma position to the Y adjacent to V includes the following:

20

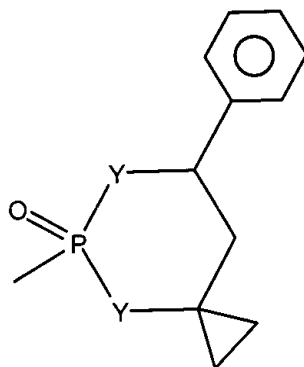


The phrase “together V and W are connected via an additional 3 carbon atoms to form an optionally substituted cyclic group containing 6 carbon atoms and substituted with one substituent selected from the group consisting of hydroxy, acyloxy, alkoxycarbonyloxy, alkylthiocarbonyloxy, and aryloxy carbonyloxy, attached to one of said additional carbon atoms that is three atoms from a Y adjacent to V includes the following:



The structure above has an acyloxy substituent that is three carbon atoms from a Y, and an optional substituent, -CH₃, on the new 6-membered ring. There has to be at least one hydrogen at each of the following positions: the carbon attached to Z; both carbons alpha to the carbon labeled “3”; and the carbon attached to “OC(O)CH₃” above.

The phrase “together W and W’ are connected via an additional 2-5 atoms to form a cyclic group, optionally containing 0-2 heteroatoms, and V must be aryl, substituted aryl, heteroaryl, or substituted heteroaryl” includes the following:

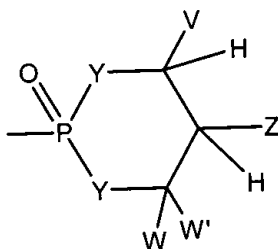


The structure above has V=aryl, and a spiro-fused cyclopropyl group for W and W'.

- 5 The term "phosphoramidite" refers to compounds attached via C, O, S, or N to the phosphorus in -P(YR)(YR) including cyclic forms, where Y is independently -O- or -NR⁶-, but where at least one Y is -NR⁶-.

- The term "phosphoramidate" refers to compounds attached via C, O, S, or N to the phosphorus in -P(O)(YR)(YR), including cyclic forms, where Y is independently -O- or -NR⁶-, but where at least one Y is -NR⁶-.
- 10

The term "cyclic phosphoramidate" refers to phosphoramidates where -P(O)(YR)(YR) is



15

The carbon attached to V must have a C-H bond. The carbon attached to Z must also have a C-H bond.

- The term "carbocyclic sugar" refers to sugar analogs that contain a carbon in place of the oxygen normally found in the sugar ring. It includes 5-membered rings such as ribofuranosyl and arabinofuranosyl sugars wherein the ring oxygen is replaced by carbon.
- 20

The term "acyclic sugar" refers to sugars that lack a ring, e.g. ribofuranosyl ring. An example is 2-hydroxyethoxymethyl in place of the ribofuranosyl ring.

The term "L-nucleoside" refers to enantiomer of the natural β -D-nucleoside analogs.

The term "arabinofuranosyl nucleoside" refers to nucleoside analogs containing an arabinofuranosyl sugar, i.e. where the 2'-hydroxyl of ribofuranosyl sugars is on the opposite face of the sugar ring.

The term "dioxolane sugar" refers to sugars that contain an oxygen atom in place of the 3' carbon of the ribofuranosyl sugar.

The term "fluorinated sugars" refers to sugars that have 1-3 carbon-fluorine atoms.

The term "P450 enzyme" refers to cytochrome enzymes that oxidize organic compounds and includes naturally occurring P450 isozymes, mutants, truncated enzymes, post-transcriptase modified enzymes, and other variants.

The term "liver" refers to liver and to like tissues and cells that contain the CYP3A4 isozyme or any other P450 isozyme found to oxidize the phosphoramidates of the invention. Based on Example F, we have found that prodrugs of formula VI and VIII are selectively oxidized by the cytochrome P450 isoenzyme CYP3A4. According to DeWaziers *et al.* (*J. Pharm. Exp. Ther.*, 253, 387-394 (1990)), CYP3A4 is located in humans in the following tissues (determined by immunoblotting and enzyme measurements):

	<u>Tissues</u>	<u>% of liver activity</u>
20	Liver	100
	Duodenum	50
	jejunum	30
	ileum	10
	colon	<5 (only P450 isoenzyme found)
25	stomach	<5
	esophagus	<5
	kidney	not detectable

Thus, "liver" more preferably refers to the liver, duodenum, jejunum, ileum, colon, stomach, and esophagus. Most preferably, liver refers to the liver organ.

The term "hepatic" refers to the liver organ cells and not cells from the duodenum, for example.

The term "enhancing" refers to increasing or improving a specific property. It includes the situation where originally there was no activity.

The term "liver specificity" refers to the ratio:

$$\frac{\text{[parent drug or a drug metabolite in liver tissue]}}{\text{[parent drug or a drug metabolite in blood, urine, or other non-hepatic tissue]}}$$

as measured in animals treated with the drug or a prodrug. The ratio can be determined by measuring tissue levels of the parent drug or drug metabolite(s) including the biologically active drug metabolite or both at a specific time or may represent an AUC based on values measured at three or more time points.

The term "increased or enhanced liver specificity" refers to an increase in the liver specificity ratio in animals treated with the prodrug relative to animals treated with the parent drug.

The term "enhanced oral bioavailability" refers to an increase of at least 50% of the absorption of the dose of the parent drug or prodrug(not of this invention) from the gastrointestinal tract. More preferably it is at least 100%. Measurement of oral bioavailability usually refers to measurements of the prodrug, drug, or drug metabolite in blood, tissues, or urine following oral administration compared to measurements following systemic administration.

The term "parent drug" refers to MH for phosphoramidates where M is connected to -P(O)(YR)(YR) via oxygen, sulfur or nitrogen and to M-PO₃²⁻ when M is connected to -P(O)(YR)(YR) via carbon. For example, AZT can be thought of as a parent drug in the form of MH. In the body AZT is first phosphorylated to AZT-PO₃²⁻ and then further phosphorylated to form AZT-triphosphate, which is the biologically active form. The parent drug form MH only applies when M is attached via N, S, or O. In the case of PMEAs, the parent drug form is M-PO₃²⁻.

The term "drug metabolite" refers to any compound produced *in vivo* or *in vitro* from the parent drug, or its prodrugs.

The term "pharmacodynamic half-life" refers to the time after administration of the drug or prodrug to observe a diminution of one half of the measured pharmacological response. Pharmacodynamic half-life is enhanced when the half-life is increased by preferably at least 50%.

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The term "pharmacokinetic half-life" refers to the time after administration of the drug or prodrug to observe a diminution of one half of the drug concentration in plasma or tissues.

5 The term "therapeutic index" refers to the ratio of the dose of a drug or prodrug that produces a therapeutically beneficial response relative to the dose that produces an undesired response such as death, an elevation of markers that are indicative of toxicity, and/or pharmacological side effects.

10 The term "enhancing the level of the biologically active drug" refers to increasing the level of the biologically active drug in the liver relative to the level achieved after administration of the parent drug where the same mode of administration is used for both the prodrug and the parent drug.

The term "cancer expressing a P450 enzyme" refers to tumor cells having a specific activity, either with or without an agent that induces the P450 activity in the tumor cells, of at least 5% of the liver specific activity.

15 The term "low levels of enzymes" refers to levels below that necessary to produce the maximal response of the biologically active agent.

The term "inadequate cellular production" refers to levels that are below that necessary to produce the maximal response of the biologically active agent. For example, the response may be viral titre.

20 The term "sustained delivery" refers to an increase in the period in which there is a prolongation of therapeutically-effective drug levels due to the presence of the prodrug.

The term "bypassing drug resistance" refers to the loss or partial loss of therapeutic effectiveness of a drug (drug resistance) due to changes in the biochemical pathways and cellular activities important for producing and maintaining the biologically active form of the drug at the desired site in the body and to the ability of an agent to bypass this resistance through the use of alternative pathways and cellular activities.

25 The term "FBPase inhibitors" refers to compounds that inhibit the human enzyme fructose 1,6-bisphosphatase with an IC₅₀ of at least 100 μ M and lower glucose in a normal 18-hour fasted rat following a 100 mg/kg dose i.v. The biologically active FBPase inhibitors are M-PO₃²⁻ wherein M is connected via a carbon, or via an oxygen when MH is a imidazole containing nucleoside analog.

The term "biologically active drug or agent" refers to the chemical entity that produces the biological effect. In this invention, biologically active agents refers to M-

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PO_3^{2-} , $\text{M-P(O)}^-\text{NHR}^{6-}$, $\text{MP}_2\text{O}_6^{3-}$, or $\text{MP}_3\text{O}_9^{4-}$ where M can be the same M as in the parent drug or a metabolite.

The term "therapeutically effective amount" refers to an amount that has any beneficial effect in treating a disease or condition.

5 The term "decreased activity of the phosphorylating enzyme" includes decreased production of the enzyme, mutations that lead to decreased efficiency (lower K_m or V_{max}), or enhanced production of endogenous inhibitors of the enzyme.

The term "phosph(on)ate" refers to compounds attached via C, O, S, or N to PO_3^{2-} or $\text{P(O)}(\text{NHR}^6)\text{O}^-$. The counterion may be H^+ or a metal cation.

10 The term "nucleoside" refers to a purine or pyrimidine base, including analogs thereof, connected to a sugar, including heterocyclic and carbocyclic analogs thereof.

The following well known drugs are referred to in the specification and the claims.

Abbreviations and common names are also provided.

15 araA; 9-b-D-arabinofuranosyladenine (Vidarabine)
AZT; 3'-azido-2',3'-dideoxythymidine (Zidovudine)
d4T; 2',3'-didehydro-3'-deoxythymidine (Stavudine)
ddI; 2',3'-dideoxyinosine (Didanosine)
ddA; 2',3'-dideoxyadenosine
ddC; 2',3'-dideoxycytidine (Zalcitabine)
20 L-ddC; L-2',3'-dideoxycytidine
L-FddC; L-2',3'-dideoxy-5-fluorocytidine
L-dT; b-L-thymidine (NV-02B)
L-dC; b-L-2-deoxycytidine (NV-02C)
L-d4C; L-3'-deoxy-2',3'-didehydrocytidine
25 L-Fd4C; L-3'-deoxy-2',3'-didehydro-5-fluorocytidine
3TC; (-)-2',3'-dideoxy-3'-thiacytidine; 2'R,5'S(-)-1-[2-(hydroxymethyl)oxathiolan-5-yl]cytosine
(Lamivudine)
1-b-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin)
30 FIAU; 1-(2-deoxy-2-fluoro-b-D-arabinofuranosyl)-5-iodouridine
FIAC; 1-(2-deoxy-2-fluoro-b-D-arabinofuranosyl)-5-iodocytosine
BHCG; (\pm)-(1a,2b,3a)-9-[2,3-bis(hydroxymethyl)cyclobutyl] guanine
FMAU; 2'-Fluoro-5-methyl-b-L-arabino-furanosyluracil
BvaraU; 1-b-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (Sorivudine)
35 E-5-(2-bromovinyl)-2'-deoxyuridine
TFT; Trifluorothymidine (Trifluorothymidine)
5-propynyl-1-arabinosyluracil (Zonavir)
CDG; carbocyclic 2'-deoxyguanosine
DAPD; (-)-B-D-2,6-diaminopurine dioxolane
40 FDOC; (-)-B-D- 5-fluoro-1-[2-(hydroxymethyl)-1,3-dioxolane]cytosine
d4C; 3'-deoxy-2',3'-didehydrocytidine
DXG; dioxolane guanosine

FEAU; 2'-deoxy-2'-fluoro-1-b-D-arabinofuranosyl-5-ethyluracil
 FLG; 2',3'-dideoxy-3'-fluoroguanosine
 FLT; 3'-deoxy-3'-fluorothymidine
 FTC; (-)-cis-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine
 5-yl-carbocyclic 2'-deoxyguanosine (BMS200,475)
 [1-(4'-hydroxy-1',2'-butadienyl)cytosine] (Cytallene)
 Oxetanocin A; 9-(2-deoxy-2-hydroxymethyl-beta-D-erythro-oxetanosyl)adenine
 Oxetanocin G; 9-(2-deoxy-2-hydroxymethyl-beta-D-erythro-oxetanosyl)guanine
 ddAPR: 2,6-diaminopurine-2',3'-dideoxyriboside
 3TC; (-)-2',3'-dideoxy-3'-thiacytidine; (2R, 5S) 1-[2-(hydroxymethyl)-1,3-oxathiolane-5-yl] cytosine (Lamivudine)
 Cyclobut A; (+/-)-9-[(1 beta,2 alpha,3 beta)-2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine
 Cyclobut G; (+/-)-9-[(1 beta,2 alpha,3 beta)-2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine (Lobucavir)
 5-fluoro-2'-deoxyuridine (Floxuridine)
 dFdC; 2',2'-difluorodeoxycytidine (Gemcitabine)
 araC; arabinosylcytosine (Cytarabine)
 bromodeoxyuridine
 IDU; 5-iodo-2'-deoxyuridine (Idoxuridine)
 CdA; 2-chlorodeoxyadenosine (Cladribine)
 F-ara-A; fluoroarabinosyladenosine (Fludarabine)
 ACV; 9-(2-hydroxyethoxymethyl)guanine (Acyclovir)
 GCV; 9-(1,3-dihydroxy-2-propoxymethyl)guanine (gancyclovir)
 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (Penciclovir)
 (R)-9-(3,4-dihydroxybutyl)guanine (Buciclovir)
 phosphonoformic acid (Foscarnet)
 PPA; phosphonoacetic acid
 PME A; 9-(2-phosphonylmethoxyethyl) adenine (Adefovir)
 PMEDAP; 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine
 HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (Cidofovir)
 HPMPA; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine
 FPMPA; 9-(3-fluoro-2-phosphonylmethoxypropyl) adenine
 PMPA; 9-[2-(R)-phosphonylmethoxypropyl] adenine (Tenofovir)
 araT; 9-b-D-arabinofuranosylthymidine
 FMdC; (E)-2'-deoxy-2'(fluoromethylene)cytidine
 AICAR; 5-aminoimidazole-4-carboxamido-1-ribofuranosyl

Detailed Description of the Invention

The invention is directed to the use of new cyclic phosphoramidate methodology which allows compounds to be efficiently converted to phosph(on)ate containing compounds by P450 enzymes found in large amounts in the liver and other tissues containing these specific enzymes. This methodology can be applied to various drugs and to diagnostic imaging agents. More specifically, the invention is directed to the use of prodrugs that undergo non-esterase-mediated hydrolysis reactions to produce MP(O)(NHR⁶)O⁻ which is either biologically active or is transformed into the biologically

active agent $M-PO_3^{2-}$, $M-P_2O_6^{3-}$ or $M-P_3O_9^{4-}$. Because highly charged phosph(on)ate containing compounds are not readily absorbed in the gastrointestinal tract, this prodrug methodology can be used to enhance absorption after oral administration.

5 In another aspect of the invention, this prodrug methodology can also be used to enhance the pharmacodynamic half-life relative to the parent drug because the cyclic phosph(on)ates of the invention can avoid metabolism by enzymes which metabolize the parent drug. Similarly, prodrugs of the invention can enhance the pharmacodynamic half-life of the phosph(on)ate-containing compound because the prodrug avoids clearance pathways used to clear negatively-charged compounds from the bloodstream.

10 In another aspect of the invention, this prodrug methodology can also be used to achieve sustained delivery of the parent drug because oxidation of the novel prodrugs proceeds in the liver at different rates and therefore selection of prodrugs that oxidize slowly but at a rate suitable for maintaining therapeutically effective levels will produce a sustained therapeutic effect.

15 The novel cyclic 1,3-propanylester methodology of the present invention may also be used to increase the distribution of a particular drug or imaging agent to the liver which contains abundant amounts of the P450 isozymes responsible for oxidizing the cyclic 1,3-propanylester of the present invention so that the free phosph(on)ate is ultimately produced. Accordingly, this prodrug technology should prove useful in the treatment of
20 liver diseases or diseases where the liver is responsible for the overproduction of the biochemical end product such a glucose, cholesterol, fatty acids and triglycerides. Such diseases include viral and parasitic infections, liver cancer, liver fibrosis, diabetes, hyperlipidemia, and obesity. In one aspect, preferably the disease is not diabetes. In addition, the liver specificity of the prodrugs should also prove useful in the delivery of
25 diagnostic agents to the liver. High levels of the biologically active drug are achieved in tissues expressing P450 enzymes capable of cleaving the prodrug due to significant retention by these cells of the activated prodrug, subsequent prodrug cleavage intermediates as well as the biologically active drug. In addition, high levels of the biologically active drug are achieved relative to the levels achieved after administration of
30 the parent drug when the parent drug is poorly phosphorylated in the tissue or has a relatively short pharmacokinetic half-life due to metabolism or other clearance mechanisms.

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The use of certain prodrug analogs of cyclophosphamide are also envisioned for treatment of cancers of the GI tract (*e.g.* primary and secondary liver cancers) as well as cancers where cytochrome P450s are introduced artificially, *e.g.* by retrovirus and other well known gene therapy strategies as well as via implanted cells engineered to express cytochrome P450s.

These specific P450 enzymes are also found in other specific tissues and cells, and thus this methodology may also be used to increase the delivery of these agents to those tissues.

In another aspect of the invention, the characteristic that most of the cyclic phosphoramidates of the present invention are metabolized in the liver to produce the phosph(on)ate can enable the use of the prodrug methodology of the present invention to increase the therapeutic index of various drugs which have side effects related to the amount of the drug or its metabolites which are distributed in extrahepatic tissues. Increased therapeutic index can also result from increased liver levels of the biologically active agent and therefore greater efficacy relative to the parent drug administered at similar doses.

In another aspect of the invention, prodrugs can be used to deliver phosph(on)ates to tissues that generate suboptimal levels of phosph(on)ates and their associated higher phosphorylated metabolites due to poor substrate activity of the parent compound and/or poor expression of enzymes used to phosphorylate the parent compound. Poor expression of the phosphorylating enzyme occurs naturally or from down regulation of the phosphorylating enzyme following chronic administration of the drug or other agents.

In another aspect of the invention, novel phosphoramidite and phosphoramidate intermediates are described.

In another aspect, methods of preparing the cyclic phosphoramidate prodrugs are described.

These aspects are described in greater detail below.

Phosphoramidate Drugs:

30 Compounds of the formula $M-P(O)(NHR^6)O^-$ are useful compounds for binding to nucleotide binding sites, such as AMP-binding sites, and certain sites known to recognize negatively charged compounds, *e.g.* carboxylic and phosphonic acids. Enzymes that catalyze the addition of water to a carbonyl compound, specifically a peptide carbonyl, an

Sub D3
5 ester, a ketone or an aldehyde, are of particular interest since these enzymes recognize compounds that have a tetrahedral group containing a negatively charged oxygen. An example is the zinc metalloproteinase class of enzymes which add water across a peptide carbonyl using a zinc-assisted catalytic mechanism. These enzymes are inhibited by phosphoramidates, e.g. NEP 24.11 is inhibited by the natural product Phosphoramidon. The prodrug strategy can provide a useful means for delivery of these compounds orally, or delivery of certain compounds to the liver in order to achieve greater efficacy or a greater therapeutic window. Enzymes inhibitors that may be suitable for delivery as prodrugs include certain phosphoramidates that inhibit NEP24.11, collagenase, stromolysin, 10 gelatinase, ACE, endothelin converting enzyme, and metalloproteinases involved in matrix remodeling such as occurs in Rheumatoid arthritis and osteoarthritis and in the heart following an acute myocardial infarction, and tumor metastasis.

Phosphonic Acids and Phosphoric Acids:

15 Cleavage of the prodrug of formula I produces the phosphoramidate M-P(O)(NHR)O⁻ which in some cases is further converted to the corresponding M-PO₃²⁻ via either chemical hydrolysis or enzyme catalyzed hydrolysis (phosphatases, amidases). The product M-PO₃²⁻ can either be the drug or itself may be further metabolized to produce higher order phosphates, e.g. M-P₂O₆³⁻ or M-P₃O₉⁴⁻ via cellular kinases. In some cases the 20 intermediate M-P(O)(NHR)O⁻ may be converted to higher order phosphates via initial conversion to M-H.

Enhancing Oral Bioavailability

25 The invention pertains to certain cyclic phosphoramidates and their use to deliver, most preferably via oral administration, a therapeutically effective amount of the corresponding phosph(on)ate compounds, preferably to an animal in need thereof. Prodrugs of the invention enhance oral bioavailability of certain drugs by changing the physical property of the drug as a consequence of the prodrug moiety and its substituents V, Z, W, and W'. The active drug may be M-P(O)(NHR⁶)O⁻ or M-PO₃²⁻. Alternatively, 30 both may instead undergo further metabolism, e.g. phosphorylation by kinases to form M-P₂O₆³⁻ and/or M-P₃O₉⁴⁻ as the active drug substance.

Compounds containing a free phosphonic acid or a phosphoric acid group generally exhibit poor (<2%) oral bioavailability since these groups are highly charged at

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physiological pH. Charged groups on compounds with molecular weights greater than 250 Daltons impede passive diffusion across cell membranes as well as absorption across the gut epithelial cell layer. Neutral prodrugs of these compounds have therefore been studied since these compounds would be more lipophilic and therefore more likely to exhibit improved intestinal permeability. Although many prodrug classes have been reported, few have been found that exhibit properties suitable for drug development.

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The most common prodrug class, and the class almost exclusively used for clinical candidates, is the acyloxyalkyl esters. These prodrugs, however, often exhibit only a modest improvement in oral bioavailability due to poor aqueous stability, poor stability to acidic/basic pH and rapid degradation by esterases in the gastrointestinal tract (Shaw & Cundy, *Pharm. Res.* 10, (Suppl), S294 (1993). Another class of prodrugs are the bis-aryl prodrugs (e.g. DeLombert *et al. J. Med. Chem.* 37, 498 (1994)) which have shown in a few isolated cases to provide good to modest improvements in oral bioavailability. The major limitation with this class of compounds is that the prodrug ester often is degraded to the monoacid rapidly in vivo but conversion to the parent drug occurs only slowly (sometimes over days), if at all.

15
The prodrugs of the invention exhibit improved properties that lead to enhanced oral bioavailability relative to the parent drug. Several characteristics of the present cyclic phosphoramidate prodrugs may contribute to their ability to enhance oral bioavailability. First, the prodrugs exhibit good stability in aqueous solutions across a wide range of pHs. (Example A) This pH stability prevents immediate hydrolysis in the mouth and GI tract prior to absorption. The pH stability can also be beneficial during formulation of the product.

20
Second, the prodrugs are resistant to esterases and phosphatases which are abundant in the gastrointestinal tract. Because much of the administered dose remains intact in the G.I. tract, the compound remains less highly charged than a free phosph(on)ate which means more of the drug can be absorbed by passive diffusion and enter the blood stream. (Example B)

25
Last, the prodrug can limit metabolism at other sites on the molecule. For example, the prodrugs of the invention eliminate metabolism of the purine base of araA by adenosine deaminase which is also abundant in the GI tract. (Example B) The amine of araA which is normally deaminated by the enzyme is protected by the cyclic phosphate moiety which is located on the 5'-ribofuranosyl hydroxyl. Reduced metabolism at other

sites of the molecule enables more of the drug to circulate in the blood stream. Although not all of these properties will be applicable to every prodrug of every drug, each of these properties can enable more drug to survive the GI tract and be available for absorption.

The novel prodrug strategy of the invention will be useful for the oral delivery of drugs that act in the liver as well as certain drugs that act on targets located in the vascular system or extrahepatic tissues. Because the highest concentration of CYP3A4 (the enzyme responsible for activating the novel prodrugs) is in the liver, the biologically active drug has a high concentration in the liver, relative to other tissues. In one aspect, parent drugs which act in the liver are preferred.

However, some of the phosph(on)ates are exported by organic anion transporters in the liver and enter the blood stream. Many phosph(on)ates in the blood stream are cleared quickly by the kidneys. Such compounds probably will not reach therapeutic levels in extrahepatic tissues. However, there are some phosph(on)ates and phosphates that are able to remain in circulation because they are not rapidly cleared by the kidneys (*e.g.* NEP inhibitors). Such compounds are able to achieve therapeutically effective levels in blood and extrahepatic tissues. Thus, in another aspect, oral delivery to extrahepatic tissues of phosph(on)ates which are not cleared by the kidneys is preferred. Thus, such parent drugs that act at sites accessible to the free phosph(on)ic acid such as targets within the vasculature system, or enzyme or receptor targets that are located on cell membranes which are exposed to the blood or fluid in the intrastitial space are preferred. Targets suitable for this aspect of the invention would be targets in which the phosphonic acid administered parenterally (*e.g.* via i.v. injection) produces a pharmacological or biochemical response expected to be useful for treating a disease condition.

Since the inhibitors exhibit poor oral bioavailability (<2%), prodrugs of the type described in this invention could enhance the oral bioavailability and produce the phosphonic acid following prodrug cleavage in the liver. Suitable circulating drug levels are expected after prodrug cleavage in the liver, since the liver is known to excrete phosphonic acids into the circulation.

Oral bioavailability can also be calculated by comparing the area under the curve of prodrug, parent drug, and/or metabolite concentration over time in plasma, liver, or other tissue or fluid of interest following oral and i.v. administration. (Example P)

For example, for drugs excreted renally in large amounts, oral bioavailability can be measured by comparing the amount of the parent drug or metabolite excreted in the

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urine, for example, after oral and i.v. administration of the prodrug. A lower limit of oral bioavailability can be estimated by comparison with the amount of parent drug excreted in the urine after administration of the prodrug (p.o.) and the parent drug (i.v.). Prodrugs of the invention show improved oral bioavailability across a wide spectrum of prodrugs, with many preferably showing increases of 1.5 to 10-fold in oral bioavailability. More preferably, oral bioavailability is enhanced by at least 2-fold compared to the parent drug.

Agents are known that inhibit CYP3A4 in the gastrointestinal tract. For example, grapefruit juice is known to decrease the activity putatively via a component in the grapefruit juice (e.g. Bergamottin; Chem Res Toxicol 1998, 11, 252-259) which results in the inactivation and/or down regulation of the enzyme. Since only GI CYP3A4 is affected, the oral absorption of the prodrugs of this invention should be enhanced. A combination of agents that inhibit, inactivate, or downregulate P450s that metabolize the prodrugs will have the effect of enhancing their absorption and thereby making more prodrug available for metabolism in the liver. The net effect of the combination would therefore be to deliver more drug to the liver after oral absorption.

Sustained Delivery

Drugs that undergo rapid elimination in vivo often require multiple administrations of the drug to achieve therapeutically-effective blood levels over a significant period of time. Other methods are also available including sustained release formulations and devices. Prodrugs that breakdown over time can also provide a method for achieving sustained drug levels. In general, this property has not been possible with the known phosph(on)ate prodrugs since either they undergo rapid hydrolysis in vivo (e.g. acyloxyalkyl esters) or very slow conversion (e.g. di-aryl prodrugs).

The cyclic phosphoramidates of the invention are capable of providing sustained drug release by providing a steady release of the drug over time. For example, most phosphates undergo dephosphorylation in vivo within minutes after systemic administration via the action of phosphatases present in the blood. Similarly, acyloxyalkyl esters of these phosphates undergo rapid esterase-mediated hydrolysis to the phosphate which then is rapidly dephosphorylated. Some prodrugs of the current invention may enable prolonged drug delivery since many of the present prodrugs are oxidized slowly over time to the phosph(on)ate in the livers (Example S). Suitably positioned, prodrug moieties can prevent or slow systemic metabolism associated with the parent drug.

Sustained delivery of the drugs is achievable by selecting the prodrugs of formula I that are hydrolyzed in vivo at a rate capable of maintaining therapeutically effective drug levels over a period of time. The cleavage rate of the drug may depend on a variety of factors, including the rate of the P450 oxidation, which is dependent on both the substituents on the prodrug moiety, the stereochemistry of these substituents and the parent drug. Moreover, sustained drug production will depend on the rate of elimination of the intermediate generated after oxidation and the rate and availability of the prodrug to the liver, which is the major site of oxidation. Identification of the prodrug with the desired properties is readily achieved by screening the prodrugs in an assay that monitors the rate of drug production in the presence of the major P450 enzyme involved in the metabolism, in the presence of liver microsomes or in the presence of hepatocytes. These assays are illustrated in Examples C, D, F, G, I, J, respectively.

It is contemplated that prodrugs of the present invention could be combined to include, for example, one prodrug which produces the active agent rapidly to achieve a therapeutic level quickly, and another prodrug which would release the active agent more slowly over time.

Improved Pharmacodynamic Half-Life

The pharmacodynamic half-life of a drug can be extended by the novel prodrug methodology as a result of both its ability to produce drug over a sustained period and in some cases the longer pharmacokinetic half-life of the prodrug. Both properties can individually enable therapeutic drug levels to be maintained over an extended period resulting in an improvement in the pharmacodynamic half-life. The pharmacodynamic half-life can be extended by impeding the metabolism or elimination pathways followed by the parent drug. For some drugs, the prodrugs of the present invention are able to avoid the metabolism or elimination pathways associated with the parent drug and thereby exist as the prodrug for extended periods in an animal. High levels of the prodrug for an extended period result in sustained production of the parent drug which can result in an improvement in the drug pharmacodynamic half-life.

An example of the ability of the prodrug class to impede metabolic pathways associated with the parent drug is shown by the araAMP prodrugs. In comparison to araAMP, prodrugs show no ara-hypoxanthine ("araH") which is the known metabolic byproduct of araA produced in *e.g.* plasma and the gastrointestinal tract after oral or

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5 A common route of elimination of phosph(on)ate drugs is via the kidneys and a transporter that recognizes anionic compounds. Complete elimination of phosphonate and phosphate containing drugs from the circulation often occurs only minutes after drug administration. The prodrugs of this invention slow the elimination of these drugs by masking the negative charge until after oxidation and hydrolysis in liver and like tissues.

Delivery of a drug to the liver with high selectivity is desirable in order to treat liver diseases or diseases associated with the abnormal liver properties (e.g. diabetes, hyperlipidemia) with minimal side effects.

20 cleavage of the prodrug (Example D).

25 by-product araH, which, as reported in the literature, is readily detected after either araA or araAMP administration. Similarly, the prodrug achieves high liver levels without production of the metabolite araH after oral administration. Since the prodrugs are cleaved by liver abundant enzymes, oral administration may enable even higher liver specificity via a first pass effect.

30 The prodrugs described in this invention can be tailored such that the elimination step is fast and therefore the product is produced near the site of oxidation, which for these prodrugs is in the liver or other P450-expressing tissue/cells.

In some cases liver specificity will be achieved most optimally using prodrugs of highly reactive drugs, which after production, act locally at a fast rate relative to diffusion out of the liver.

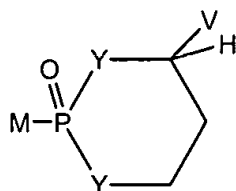
Agents that induce P450 activity, e.g. CYP3A4 activity, are known. For example, rifampicin, glucocorticoids, phenobarbital, erythromycin are known to enhance CYP3A4 activity in rat and human livers following. P450 activity can be monitored in humans by non-invasive methods e.g. via [14C] erythromycin breath test. These studies are useful in the identification of agents that activate CYP3A4 in humans. Accordingly, for prodrugs where drug delivery is limited by prodrug metabolism rate (e.g. rate of clearance of prodrug is fast relative to rate of prodrug cleavage), agents such as rifampicin can be used in combination or as adjuncts or pre-treatments to enhance CYP3A activity in the liver and thereby to increase liver drug levels. (Examples J and O)

Prodrug Cleavage Mechanism

The prodrugs of the current invention are simple, low molecular weight modifications of the drug which enable liver-selective drug delivery on the basis of the their sensitivity to liver-abundant enzymes. The prodrug cleavage mechanism is postulated to entail an oxidation and β -elimination based on studies analyzing reaction requirements and products. In some cases, $M-P(O)(NHR^6)O^-$ is further metabolized to $M-PO_3^{2-}$ and $M-P_3O_9^{4-}$. Prodrugs are stable to aqueous solution across a broad pH range and therefore do not undergo a chemical cleavage process to produce the parent drug. In addition the prodrugs are stable to esterases and blood proteins. In contrast, the prodrugs are rapidly cleaved in the presence of liver microsomes from rats (Example C) and humans (Examples D and F). The drug is also produced in freshly isolated rat hepatocytes where it is detected as the corresponding phosph(on)ate and/or biologically active agent (Examples G and J). Moreover, when the parent drug is an FBPase inhibitor, the production of the drug is supported by the ability of the prodrug to result in potent gluconeogenesis inhibition (Example I).

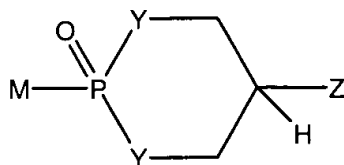
Possible specific enzymes involved in the cleavage process were evaluated through the use of known cytochrome P450 inhibitors (Example E). The studies indicate that the isoenzyme cytochrome CYP3A4 is responsible based on ketoconazole inhibition of drug formation.

The biologically active agent is detected in the liver following administration of drugs of formulae VI-VIII, shown below:

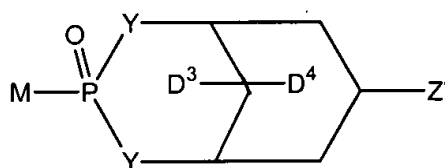


VI

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VII

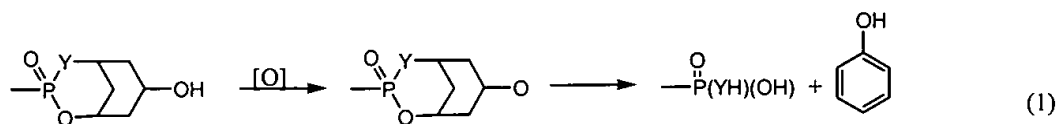


VIII

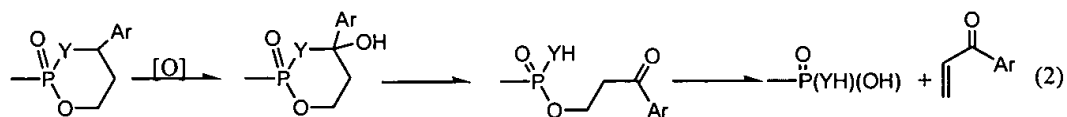
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Prodrugs of the following formulas are particularly preferred.

Prodrugs of formula VI cleave to generate aryl vinyl ketone whereas prodrugs of formula VIII cleave to generate phenol (Example L). The mechanism of cleavage could proceed by the following mechanisms, shown for compounds where one Y is NR⁶.



(1)



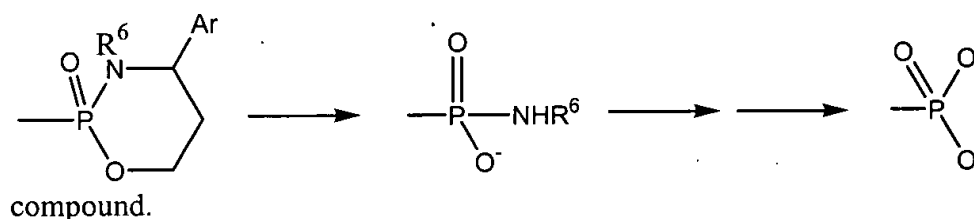
(2)



(3)

Although the esters in the invention are not limited by the above mechanisms, in general, each ester contains a group or atom susceptible to microsomal oxidation (e.g. alcohol, benzylic methine proton), which in turn generates an intermediate that breaks
 5 down to the parent compound in aqueous solution via β -elimination of the $M-P(O)(YH)_2$. This species is either the active component or is further transformed via chemical or enzymatic processes to $M-PO_3^{2-}$, $M-P_2O_6^{3-}$, or $M-P_3O_9^{4-}$. Furthermore, although these specific prodrugs are cleaved by CYP3A4, other prodrugs in the class may be substrates for other P450s. Small changes in structure are known to influence substrate activity and
 10 P450 preference. The identification of the isoenzyme(s) activating the prodrug is accomplished according to the procedure described in Example B.

Alternatively, cyclic phosphoramidates can serve as a prodrug of phosph(on)ates or higher order phosph(on)ates since intermediate phosphoramidates can be converted to MPO_3^{2-} , $MP_2O_6^{3-}$, or $MP_3O_9^{4-}$ via the action of phosphatases/amidases which can produce
 15 MH or MPO_3^{2-} which in turn can be phosphorylated by kinases to the biologically active



Increased Therapeutic Index

The prodrugs of this invention can significantly increase the therapeutic index
 20 (“TI”) of certain drugs. In many cases, the increased TI is a result of the high liver specificity. For example, araA and araAMP are known to produce significant systemic side effects and these side effects are associated with blood levels of the araA byproduct araH. Presumably the side effects are a result of toxicities of araH or araA in extrahepatic tissues (e.g. nerves) which produce e.g. the neuropathies associated with the drug in man
 25 (>40% of patients receiving araA). Prodrugs of araA show a substantial shift in the liver (araATP)/urine (araH) ratio in comparison with araAMP.

Renal toxicity is a common toxicity associated with phosphonic acids. The toxicity results from transport, e.g. via the organic anion transporters located on the basolateral membrane of the renal proximal tubule, of the negatively charged drug into

e.g. tubular cells which then accumulate the drug to high concentrations unless there is an equally efficient transport of the drug out of the cell via luminal transport mechanisms (e.g., anion exchange or facilitated diffusion). Many examples have been reported in the literature of nephrotoxic phosphonic acids, e.g. PME_A and HPMPA. The novel prodrug of PME_A shows only small amounts of PME_A in the urine relative to either PME_A or bisPOM PME_A at doses that achieved comparable liver drug levels.

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Another common toxicity associated with phosphonic acid drugs is gastrointestinal toxicity via in some cases GI erosions. Prodrugs of the current invention can decrease GI toxicities, especially toxicities produced by direct action of the drug on the GI tract after oral administration. Similar to the kidney, gut epithelial cells have organic anion transporters which can result in high intracellular drug levels and cytotoxicity. Since the negatively charged phosph(on)ate is not revealed until after absorption and cleavage in the liver, prodrugs of this invention reduce gut toxicity.

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Severe toxicities are also associated with nearly all anticancer agents. In an effort to decrease these toxicities during treatment of primary or secondary liver cancers, drugs are sometimes administered directly into the portal artery in order to increase liver drug exposure. Since oncolytic drugs typically are associated with significant side effects, local administration enables greater hepatic uptake and thereby decreased extrahepatic toxicities. To further increase liver uptake, chemoembolization is sometimes used in conjunction with hepatic artery drug infusion. The high liver specificity of the prodrugs in the current invention suggest that systemic side effects will be similarly minimized by the novel prodrug approach.

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Moreover, primary and secondary liver cancers are particularly resistant to both chemotherapy and radiotherapy. Although the mechanism for the resistance is not completely understood, it may arise from increased liver gene products that lead to rapid metabolism and/or export of chemotherapeutic agents. In addition, the liver, which is generally associated with xenobiotic metabolism and generation of cytotoxic intermediates, is equipped by nature with multiple protective mechanism so that damage from these intermediates are minimized. For example, the intracellular concentration of glutathione is very high in the liver relative to other tissues presumably so that intermediates capable of alkylating proteins and DNA are detoxified through a rapid intracellular reaction. Consequently, the liver may be resistant to chemotherapeutic agents because of these mechanisms and therefore require higher than normal concentrations of

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5 For example, the high liver specificity can enable higher doses since the dose-limiting extrahepatic side effects are reduced. Second, sustained delivery of the prodrug can enable high drug levels to be maintained over a sustained period. Third, in some cases, the parent drug is poorly phosphorylated in the target cells. The prodrugs of this invention can bypass this step and deliver the phosph(on)ate which in some cases is further
10 metabolized to the biologically active agent. Last, the therapeutic index can sometimes be further enhanced by co-administration of CYP inducers (*e.g.* rifampicin, glucocorticoids, phenobarbital, erythromycin) which can enhance conversion in the liver and thereby enable higher levels of the biologically active drug without increasing the dose (Examples J and O). These benefits of the prodrugs enable successful therapy of liver diseases, such
15 as primary and secondary liver cancers as well as other liver diseases.

Prodrugs of the invention are also useful because they generate byproducts that are either non-toxic or rapidly detoxified. Some of the byproducts generated by prodrugs of the invention are not considered to be highly toxic at doses expected to be used in the clinic, especially given that the byproduct would be generated in the liver (*e.g.* phenol). Other byproducts generated by the prodrugs of this invention, *e.g.* aryl vinyl ketone, can be considered as alkylating agents and therefore could produce either or both cytotoxicity and genotoxicity. These toxicities, however, are eliminated for prodrugs activated in the liver since: 1) the byproduct is produced at or near the site of prodrug activation; and 2) natural defense mechanisms exist at the site of byproduct generation and are able to completely detoxify the byproduct. Other mechanisms may also assist in the detoxification of the byproduct, including reactions that lead to oxidation, reduction or transformation (*e.g.* sulfation) of the byproduct.

30 The primary defense system expected to protect cells from the prodrug byproduct involves the natural thiol glutathione and possibly an enzyme that catalyzes the addition of glutathione to electrophiles, *i.e.* glutathione S-transferase (GST). Glutathione and GST are always co-expressed with CYPs as part of a natural defense mechanism that protects the

cell from highly reactive oxygen species generated during CYP-mediated oxidation of organic compounds. CYPs are expressed in certain cells (primarily hepatocytes) to oxidize organic compounds and thereby assist in their elimination from the body. Tissue distribution studies show that CYP3A4 as well as most other P450s are expressed highest in the liver followed by the small intestine. All other tissues have <<2% of the liver CYP3A4 activity. Similarly, analysis of glutathione levels indicates that the liver (10 mM) and GI have nearly 1000-fold higher glutathione levels than other tissues. These levels of glutathione are still high even in diseased livers.

Glutathione reacts rapidly with vinyl ketones, and any alkylating agent, to generate a conjugate which is then eliminated via the bile or kidney. Alkylation of other proteins or nucleic acids in the cell is not expected in cells with glutathione since glutathione is a much better nucleophile than heteroatoms on the bases of nucleic acids and exists at much higher concentration than thiol groups on the surface of proteins.

By products such as acrolein can produce bladder toxicity which these prodrugs can prevent by their ability to generate the byproduct within the hepatocyte. Examples M and N are useful for testing for byproduct toxicity, which is not expected in the liver as long as hepatic glutathione levels remain >20% of normal. Glutathione is readily measured in hepatocytes as well as *in vivo* (Examples M and N). Cytotoxicity is detected in hepatocytes via testing of cell viability with trypan blue and by analyzing for elevation of live enzymes. *In vivo*, liver toxicity is evaluated by analysis of liver enzyme elevation in the plasma.

Non-Mutagenic Prodrugs

Prodrugs of the invention are elevated by a postulated mechanism involving an initial oxidation followed by a β -elimination reaction. In some cases, e.g. certain prodrugs of formula VI and formula VII, the biproduct of the reaction is an α,β -unsaturated carbonyl compound, e.g. vinyl phenyl ketone for prodrugs where V= Ph, Z, W and W' = H. Compounds can act as Michael acceptors and react with nucleophiles via a Michael addition. Mutagenesis is observed with some α,β -unsaturated ketones and certain toxicities arise from Michael addition adducts (e.g. acrolein produces bladder toxicities). The degree to which these activities limit the use of compounds of Formula VI is dependent on the severity of the toxicity and the indicated disease.

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Prodrugs that produce non-toxic and non-mutagenic biproducts are especially preferred for the treatment of chronic diseases (e.g. diabetes). Frequently, it is difficult to predict the mutagenic properties of a compound. For example, a number of acrylates have been shown to produce positive mutagenic responses as indicated by increased

5 chromosome aberrations and micronucleus frequencies in cultured L5179Y mouse lymphoma cells (Dearfield *et al.*, *Mutagenesis* 4, 381-393 (1989)). Other acrylates, however, are negative in this test (*J. Tox. Envir. Health*, 34, 279-296 (1991)) as well as in the Ames test and the CHO assay which measures newly induced mutations at the hypoxanthine-guanine phosphoribosyltransferase (*hgpert*) locus (*Mutagenesis* 6, 77-85

10 (1991)). Phenyl vinyl ketone lacks teratogenic activity in rat embryos in culture suggesting that it may not be mutagenic nor highly toxic (*Teratology* 39, 31-37 (1989)).

Since mutagenicity and toxicity are not highly predictable properties, non-mutagenic prodrugs of formula I and their associated by-products can be readily identified by conducting well known in vitro and in vivo assays. For example, compounds can be

15 tested in non-mammalian cell assays such as the Ames test, a fluctuation test in *Kl. pneumoniae*, a forward mutation assay with *S. typhimurium*, a chromosome loss assay in *Saccharomyces cerevisiae*, or a D3 recombinogenicity assay in *Saccharomyces cerevisiae*. Compounds can also be tested in mammalian cell assays such as the mouse lymphoma cells assay (TK+/- heterozygotes of L5178Y mouse lymphoma cells), assays in

20 Chinese hamster ovary cells (e.g. CHO/HGPRT assay), and an assay in rat liver cell lines (e.g. RL1 or RL4). Each of these assays can be conducted in the presence of activators (e.g. liver microsomes) which may be of particular importance to these prodrugs. By conducting these assays in the presence of the liver microsomes, for example, the prodrug produces products, such as phenol or vinyl ketone. The mutagenicity of the by-product is

25 measured either directly or as a prodrug where the results are compared to the parent drug alone. Assays in liver cell lines are a preferred aspect of the invention since these cells have higher glutathione levels, which can protect the cell from damage caused by a Michael acceptor, as well as greater levels of intracellular enzymes used to detoxify compounds. For example, the liver contains reductases that with some by-products might

30 result in reduction of the carbonyl.

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A variety of end points are monitored including cell growth, colony size, gene mutations, micronuclei formation, mitotic chromosome loss, unscheduled DNA synthesis, DNA elongation, DNA breaks, morphological transformations, and relative mitotic activity.

5 *In vivo* assays are also known that assess the mutagenicity and carcinogenicity of compounds. For example, a non-mammalian *in vivo* assay is the *Drosophila* sex-linked recessive lethal assay. Examples of mammalian *in vivo* assays include the rat bone marrow cytogenetic assay, a rat embryo assay, as well as animal teratology and carcinogenicity assays.

10 Kinase Bypass

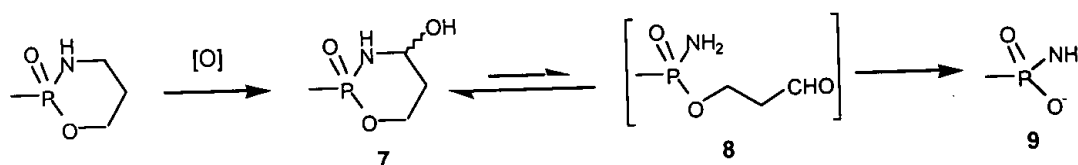
 Nucleosides are often converted in cells to mono-, di- and tri-phosphates with the latter usually acting as the biologically active species. In some cases, the triphosphate is a potent inhibitor of the target enzyme but poor to modest efficacy is achieved *in vivo* because administration of the nucleoside fails to achieve sufficiently high levels of the biologically active phosphate. Often the failure to achieve high levels of the phosphate is because the nucleoside is a poor substrate of the enzymes that catalyze the phosphorylation reaction. Other reasons include the low natural levels of the phosphorylating enzyme in the target cell which may represent low natural expression levels or be a result of chronic therapy with the drug or another drug which results in enzyme down regulation. In some cases, drug resistance results from a decrease in activity of the enzymes responsible for synthesis of a nucleoside monophosphate (e.g. kinases such as thymidylate kinase or enzymes in the biosynthesis pathway of 5-fluoro-2'-deoxy UMP). In other cases nucleoside transporters are downregulated leading to lower intracellular nucleoside drug concentration and therefore less nucleoside is available for phosphorylation. Similarly, increased expression of multidrug resistant gene product is postulated to increase the export of nucleotides from cancer cells. Administration of the prodrug generates the monophosphate by a different pathway avoiding the pathways that cause the resistance to the parent drug. Thus, the prodrugs of the present invention can achieve a therapeutic effect in cells resistant to the parent drug. Some drugs as the mono- or triphosphate analogs are highly potent inhibitors of the target enzyme (e.g. HBV polymerase) but are poorly effective in cells or *in vivo* due to poor phosphorylation. (Example H) These drugs are especially preferred drugs since the prodrug strategy

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delivers the monophosphate. Frequently, the first phosphorylation of the nucleoside is the rate-limiting step whereas phosphorylation of the monophosphate to the triphosphate by mammalian kinases is rapid and relatively insensitive to structural variations.

5 Ring-Substituted Cyclophosphamide Analogs for Treatment of Certain Cancers:

Cyclophosphamide (CPA) and Ifosfamide (IF) are commonly used oncolytic drugs that undergo initial activation in the liver via a P450 mechanism (CYP2B6 and CYP3A4) to intermediate 7. This intermediate exists in equilibrium with the ring-opened species (8), which is the penultimate intermediate for a variety of metabolic pathways, including a β -elimination reaction that generates the alkylating mustard 9 and acrolein. The latter reaction sequence occurs via a non-enzymatic reaction and is thought to proceed with a $t_{1/2}$ of $\approx 30 - 40$ minutes at neutral pH.



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Intermediates 8 and 9 are relatively lipophilic and long-lived. As a consequence, a significant proportion of the activated CPA “escapes” the liver and enters the general circulation. Both the oncolytic activity and the drug-associated side effects are due to extra-hepatic breakdown. Uptake of the intermediate by tumor cells and generation of the mustard results in inhibition of cell proliferation. Similarly, myelosuppression and decreased white cells are side effects associated with CPA therapy that result from inhibition of extra-hepatic cell proliferation.

CPA and IF are under investigation in conjunction with methods useful for the introduction of cytochrome P450s artificially, e.g. by retrovirus and other well known gene therapy strategies as well as via implanted cells engineered to express cytochrome P450s for the treatment of cancer. The hope is that with cells that express the CYP near the tumor, a more local and therefore a potentially more beneficial therapy is possible. Prodrugs of this invention have the advantage for treating primary and secondary liver cancers as well as for tumors with enhanced P450 activity since they generate the active oncolytic agent more locally, i.e. with less “escape” from the tissue containing the P450

activity. The reasons for the greater local effect are due to differences in the prodrug cleavage intermediates which include: (1) a ring-opening reaction that is rapid and irreversible; and (2) the ring-opened product, which can be negatively charged (M-P(O)(NHR⁶)O⁻) and therefore unable to exit the cell via passive diffusion.

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Types of Parent Drugs

Various kinds of parents drugs can benefit from the prodrug methodology of the present invention. Parent drugs of the form MH, which are phosphorylated to become the biologically active drug are well suited for use in the prodrug methodology of the present invention. There are many well known parent drugs of the form MH which become biologically active via phosphorylation. For example, it is well known that antitumor and antiviral nucleosides are activated through phosphorylation. These compounds include LdC, LdT, araA; AZT; d4T; ddI; ddA; ddC; L-ddC; L FddC; L-d4C; L-Fd4C; 3TC; ribavirin; 5-fluoro-2'-deoxyuridine; FIAU; FIAC; BHCG; L FMAU; BvaraU; E-5-(2-bromovinyl-2' deoxyuridine; TFT; 5-propynyl-1 arabinosyluracil; CDG; DAPD; FDOC; d4C; DXG; FEAU; FLG; FLT; FTC; 5-yl-carbocyclic 2'deoxyguanosine; oxetanocin A; oxetanocin G; Cyclobut A, Cyclobut G; dFdC; araC; bromodeoxyuridine; IDU; CdA; FaraA; Coformycin, 2'-deoxycoformycin; araT; tiazofurin; ddAPR; 9-(arabinofuranosyl)-2,6 diaminopurine; 9-(2'-deoxyribofuranosyl)-2,6 diaminopurine; 9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine; 9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine; 9-(2'-deoxy 2'fluororibofuranosyl)guanine; FMdC; 5,6 dihydro-5-azacytidine; 5-azacytidine; 5-aza 2'deoxycytidine; AICAR; ACV, GCV; penciclovir; (R)-9-(3,4 dihydroxybutyl)guanine; cytallene; PMEa, PMEDAP, HPMPC, HMPMA, FMPMA, and PMPA.

In one preferred aspect, the compounds of formula I does not include compounds where MH is araA or 5-fluoro-2'-deoxyuridine.

Preferred antiviral drugs include:

LdC, LdT, araA; AZT; d4T; ddI; ddA; ddC; L-ddC; L FddC; L-d4C; L-Fd4C; 3TC; ribavirin; FIAU; FIAC; BHCG; L-FMAU; BvaraU; E-5-(2 bromovinyl-2'-deoxyuridine; TFT; 5-propynyl 1-arabinosyluracil; CDG; DAPD; FDOC; d4C; DXG; FEAU; FLG; FLT; FTC; 5-yl-carbocyclic 2'deoxyguanosine; cytallene; oxetanocin A; oxetanocin G; Cyclobut A, Cyclobut G; bromodeoxyuridine; IDU; araT; tiazofurin; ddAPR; 9-(arabinofuranosyl)-2,6 diaminopurine; 9-(2'-deoxyribofuranosyl)-2,6

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araA; AZT; d4T; ddI; ddA; ddC; L-ddC; L-FddC; L-d4C; L-Fd4C; 3TC; ribavirin; FIAU; FIAC; L-FMAU; TFT; CDG; DAPD; FDOC; d4C; DXG; FEAU; FLG; FLT; FTC; 5-yl carbocyclic 2'deoxyguanosine; cytallene; oxetanocin A; oxetanocin G; Cyclobut A, Cyclobut G; araT; ACV, GCV; penciclovir; PMEA; PMEDAP; HPMPC; HPMPA; PMPA; foscarnet.

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are antivirals. Certain antibiotics might be suitable, especially antibiotics such as D-alanine racemase inhibitors and fosfomycin and associated analogs.

The following compounds and their analogs can be used in the prodrug methodology of the present invention:

5 **NEP Inhibitors**

(S)-3-[N-[2- [(phosphonomethyl)amino]-3-(4-biphenyl)propionyl]amino]propionic acid by DeLombaert *et al.* in *J Med Chem.* 1994 Feb 18;37(4):498-511

Collagenase Inhibitors

10 N,[N-((R)-1-phosphonopropyl-(S)-leucyl)-(S)-phenylalanine N-methyl amide by Bird *et al.* in *J Med Chem* 1994 Jan 7;37(1):158-69

Angiotensin Converting Enzyme Inhibitors

15 (IR)-1-(N-(N-acetyl-L-isoleucyl)-L-tyrosyl)amino-2-(4-hydroxyphenyl)ethyl phosphonic acid by Hirayama *et al.* in *Int J Pept Protein Res* 1991 Jul;38(1):20-4.

Endothelin Inhibitor

CGS 26303 by DeLombaert *et al.*, *Biochem Biophys Res Commun* 1994 Oct 14;204(1):407-12

20 (S, S)-3-Cyclohexyl-2-[[5-(2, 4-difluorophenyl)-2-[(phosphonomethyl)amino]pent-4-ynoyl]amino] propionic acid by Wallace *et al.*, *J Med Chem* 1998 Apr 23;41(9):1513-23

25 (S, S)-2-[[5-(2-fluorophenyl)-2-[(phosphonomethyl)amino]pent-4-ynoyl]amino]-4-methylpentanoic acid

(S, S)-2-[[5-(3-fluorophenyl)-2-[(phosphonomethyl)amino]pent-4-ynoyl] + ++amino]-4-methylpentanoic acid

30 **NMDA/AMPA Antagonists**

N-phosphonoalkyl-5-aminomethylquinoxaline-2,3-diones as described in *Bioorg Med Chem Lett.* 1999 Jan 18;9(2):249-54

35 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid by Bespalov *et al.* in *Eur J Pharmacol* 1998 Jun 26;351(3):299-305

[2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-ethyl]phosphonic acid
D,L-(E)-2-amino-4-[3H]-propyl-5-phosphono-3-pentenoic acid

40 6,7-dichloro-2(1H)-oxoquinoline-3-phosphonic acid by Desos *et al.* in *J Med Chem.* 1996 Jan 5;39(1):197-206.

cis-4-(phosphonomethyl)piperidine-2-carboxylic acid (CGS 19755)

45 **Purine Nucleoside Phosphorylase Inhibitors**

[7-(2-amino-1,6-dihydro-6-chloro-9H-purin-9-yl)-1,1-difluoroheptyl]phosphonic acid and

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[4-(5-amino-6,7-dihydro-7-oxo-3H-1,2,3,-triazolo[4,5-d]-pyrimidin-3-yl)butyl]phosphonic acid.

5 [[[5-(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)pentyl]phosphinico]methyl]phosphonic acid by Kelly *et al.* in *J Med Chem* 1995 Mar 17;38(6):1005-14

(2-[2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methyl]-phenyl]ethenyl)-phosphonic acid by Weibel *et al.* in *Biochem Pharmacol.* 1994 Jul 19;48(2):245-52.

10 9-(3,3-Dimethyl-5-phosphonopentyl)guanine by Guida *et al.* in *J Med Chem* 1994 Apr 15;37(8):1109-14.

Alanine Racemase Inhibitors

15 DL-(1-Amino-2-propenyl)phosphonic acid by Vo-Quang *et al.* in *J Med Chem* 1986 Apr;29(4):579-81

Squalene Synthase Inhibitors

20 1-Hydroxy-3-(methylpentylamino)-propylidene-1,1-bisphosphonic acid by Amin *et al.* in *Arzneimittelforschung.* 1996 Aug;46(8):759-62.

BMS188494 is POM prodrug of BMS 187745 by Dickson *et al.* in *J Med Chem.* 1996 Feb 2;39(3):661-4.

Treatment of Cancer:

25 The prodrug strategy in the current invention encompasses several features that are advantageously used in cancer therapies. Many of the known anticancer drugs are nucleosides that undergo phosphorylation to the monophosphate which frequently is further converted to the triphosphate. The prodrug strategy increases the effectiveness of these drugs because the prodrug is cleaved by liver-abundant enzymes and therefore
30 higher levels of the active metabolite are achieved in the liver relative to extrahepatic tissues. The net effect is greater efficacy and/or a greater therapeutic index. In some cases, the prodrug strategy also increases the effectiveness of these drugs by bypassing well-known resistance mechanisms including mechanisms involved in the uptake, intracellular biosynthesis of the active metabolite, export and metabolism of the
35 monophosphate. Examples of preferred drug candidates that are specifically amenable to the strategy include, e.g. dFdC, araC, F-ara, and CdA, and 5-fluoro-2'-deoxyuridine ("5-FU"). In one aspect, MH is not 5-FU.

Some prodrugs may result in some accumulation of the monophosphate in cells. Certain monophosphates are useful for the treatment of cancers, e.g. monophosphates that
40 are potent inhibitors of thymidylate synthase (TS) and IMP dehydrogenase. Some TS inhibitors are reported to be moderately effective in treating liver cancers. For example, 5-

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5 FU and 5-FdUMP are effective. These drugs, however, are plagued by drug resistance and severe side effects. To avoid the latter, 5-FU analogs are often delivered via the portal artery to achieve the highest possible liver levels. Accordingly, 5-FdUMP and associated analogs are suitable targets for the prodrug strategy. Other nucleosides such as ribavirin, tiazofurin and coformycin and analogs thereof are also suitable targets for the prodrug strategy.

10 Liver cancers are also very resistant to radiotherapy. One method for enhancing radiotherapy is to administer radiosensitizers. Radiosensitizers such as 2'-deoxy-5-iodouridine are nucleosides suitable for the prodrug technology since the prodrugs will be cleaved by only P450 containing cells. After cleavage the monophosphate can be further phosphorylated to the triphosphate and trapped inside the cell making the cell more sensitive to radiotherapy.

Treatment of Viral Infections:

15 Drugs useful for treating viruses that infect the liver and cause liver damage, e.g. hepatitis virus strains, exhibit similar properties to the anticancer nucleoside drugs in terms of efficacy, side effects and resistance. Prodrugs of these nucleosides would therefore be useful in treating hepatitis. In some cases, the drugs are already targeted for hepatitis (e.g. araA, 3TC, L-FMAU, FTC, BMS 200,475). The prodrugs of these compounds could
20 enhance the efficacy, increase the therapeutic index, improve the pharmacodynamic half-life and/or bypass drug resistance. Prodrugs of other agents used to treat viral infections other than hepatitis may also be made useful by administration of the prodrugs of this invention since these drugs are good antivirals (e.g. acyclovir, but useful for other viral infections because they are phosphorylated to the monophosphono by a viral kinase. The
25 monophosphate is converted to the biologically active triphosphate by mammalian kinases. Accordingly, delivery of the monophosphate using this clan of prodrugs enables treatment of hepatitis by drugs normally used to treat other viral infections.

Use for the delivery of diagnostic agents:

30 Nucleoside diagnostic agents, e.g. uridine analogs with the 5H substituted with Tc, are useful as the prodrugs of this invention as liver diagnostic agents. These compounds are converted first to the monophosphate and then onto the triphosphate in cells that contain P450 activity, specifically CYP3A4 activity. Since nearly all of the activity is in

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the liver, diagnostic agents of this type will primarily be metabolized in the liver and accumulate in cells that metabolize the prodrug. Accordingly, liver tumors that have no CYP3A4 activity or tumors such as hepatocellular carcinomas which have approximately 50% of the normal activity may be differentiated from normal liver tissue.

5

Agents Used to Modulate CYP Activity

A variety of methods may be used to enhance the in vivo activity of compounds of formula I. For example, various drugs are known that enhance cytochrome P450 (CYP) activity. Enhancement frequently entails increased gene transcription. Four families of CYPs are particularly susceptible to induction, namely CYP1-4. Induction is purportedly via receptors that are activated by various xenobiotics. For example, CYP1 gene activation frequently involves activation of the Ah receptor by polycyclic aromatic hydrocarbons. CYP2-4 are activated via orphan nuclear receptors. Data suggests that the nuclear receptor CAR (constitutively Active Receptor) is responsible for phenobarbital CYP activation, especially CYP2 genes. The pregnane nuclear receptors (PXR or PAR or SXR) are thought to activate CYP3A genes whereas the PPAR (peroxisome proliferator activate receptor) is linked to CYP4 gene activation. All three xenobiotic receptors are highly expressed in the liver which accounts for the liver specificity of the P450 gene induction.

Xenobiotics known to induce CYP3 genes include phenobarbital, a variety of steroids, e.g. dexamethasone, antibiotics, e.g. rifampicin, and compounds such as pregnenolone-16a carbonitrile, phenytoin, carbamazepine, phenylbutazone, etc. A variety of methods are known that enable identification of xenobiotics that induce P450s, including a reporter gene assay in HepG2 cells (Ogg *et al.*, *Xenobiotica* 29, 269-279 (1999). Other inducers of the CYP3A subfamily are known that act at the post-transcriptional level either by mRNA or protein stabilization, e.g. clotrimazole, TA and erythromycin. Compounds known to induce CYPs or identified in in vitro assays are then used to enhance CYP activity in vivo. For example, CYP activity is monitored in rats pre-treated with CYP modulators by e.g. evaluating liver microsomes over a period of time to determine the optimal pre-treatment period, dose and dosing frequency. Rats with enhanced CYP activity, especially the CYP activity responsible for activation of the prodrugs (e.g. CYP3A4), are then treated with compounds of formula 1. Enhanced CYP activity can then lead to enhanced prodrug conversion and liver specificity. For example,

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enhanced metabolism of cyclophosphamide was found with pre-treatment with phenobarbital (Yu *et al.*, *J. Pharm. Exp. Ther.* 288, 928-937 (1999)).

In some cases, enhanced CYP activity may lead to unwanted drug metabolism. For example, enhanced activity of CYPs not involved in prodrug activation can result in increased drug metabolism and therefore decreased efficacy. In addition, increased CYP activity in other tissues, e.g. CYP3A4 in the gastrointestinal tract, could result in decreased prodrug absorption and liver drug levels. Inhibitors of CYP activity are known that might be useful in minimizing unwanted drug metabolism. For example, grapefruit juice is known to inactivate gastrointestinal CYP3A4 and to result in enhanced absorption of numerous drugs metabolized by CYP3A4. CYP inhibitors are also known for many of the CYP subfamilies that can be useful for attenuating unwanted drug metabolism while maintaining CYP activity important for prodrug cleavage. For example, the CYP3A inhibitor TAO was used to modulate cyclophosphamide metabolism in vivo in a manner that decreased the formation of toxic metabolites that do not contribute to its antitumor activity.

Methods for Monitoring Patient P450 Activity

CYP activity is known to exhibit significant differences across individuals. The range for CYP3A4 is 5- to 20-fold although most individuals are within a 3-fold range. Modest decreases are noted for individuals with liver disease (30-50%) or advanced age (25-50%). Differences for gender are even more modest (<25%). Methods for phenotyping an individual's CYP activity are known and could be useful in predicting who should receive drugs that modulate CYP activity. Evasive procedures include liver biopsy. Non evasive procedures have been reported, including an "erythromycin breath test" which is based on the exhalation of $^{14}\text{CO}_2$ generated from the CYP3A-mediated N-demethylation of radiolabeled erythromycin (iv). (Watkins, *Pharmacogenetics* 4, 171-184 (1994)).

Gene Therapy

Introduction into tumor cells genes that encode for enzymes not normally expressed represents a new therapeutic strategy for increasing the therapeutic effectiveness of anticancer chemotherapies. The general strategy entails expression of an enzyme that catalyzes the breakdown of a prodrug of an anticancer drug thereby localizing the drug in or near the tumor mass and limiting exposure elsewhere. The strategy has been

demonstrated using the HSV-TK gene wherein the thymidylate kinase specifically expressed in the transfected cells activates ganciclovir to the monophosphate which is then converted by other kinases to the tumor cell killing triphosphate. A similar strategy uses the bacterial cytosine deaminase gene for conversion of 5-fluorouracil to 5-fluorocytosine.

5 Other genes have been considered including carboxypeptidase G2, nitro reductase, purine nucleoside phosphorylation, etc. In addition, CYP gene transfer has been explored as a way to enhance the chemotherapeutic effect of cyclophosphamide and ifosfamide, two drugs known to be activated by CYPs. For example, human breast cancer cells were sensitized by transfection with the CYP2B1 gene (Chen *et al.*, *Cancer Research*, 56, 1331
10 1340 (1996)). The advantage of this strategy relative to the HSV-TK gene strategy is that the product of the CYP catalyzed oxidation readily diffuses outside of the tumor cell and into nearby cells. In contrast to monophosphate products of the HSV-TK strategy, the product can enter cells that are not in cell-cell contact and therefore produce a more widespread tumor killing effect (Chen and Waxman, *Cancer Research*, 55, 581-589
15 (1995)).

Compounds of formula 1 can be made more effective by using gene therapy to introduce the gene that encodes the CYP specifically involved in prodrug cleavage. The specific CYP that breaks down the prodrug is readily determined using some or all of the following steps: 1) demonstrate prodrug cleavage using human microsomes; 2) classify
20 the subfamily by comparing activity with microsomes induced with various subfamily specific inducers (e.g. CYP3 enzymes are induced with a variety of steroids, e.g. dexamethasone, antibiotics, e.g. rifampicin, and compounds such as pregnenolone-16a carbonitrile, phenytoin, carbamazepine, phenylbutazone, etc.; 3) identify the CYP or CYPs responsible for prodrug activation by using known CYP subfamily specific inhibitors (e.g.
25 troleandomycin, erythromycin, ketoconazole and gestodene) and/or by using neutralizing antibodies; 4) confirm CYP subfamily by demonstrating turnover via the recombinant enzyme.

Genes are introduced to the tumor using a suitable vector (e.g. retroviral vectors, adenoviral vectors) or via direct DNA injection. P450 activity can also be introduced into
30 or near the tumor mass using cells engineered to express P450s. Preferred are encapsulated cells (e.g. Lohr *et al.*, *Gene Therapy* 5: 1070 (1998)) The compounds of formula I are then introduced following significant enhancement of the CYP activity in the tumor.

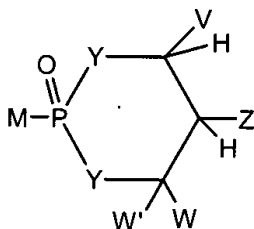
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Especially preferred are those prodrugs disclosed in the invention that are converted to the parent phosph(on)ate in cells and tissues, especially hepatocytes and liver, as indicated by measurement of the intracellular drug metabolites in hepatocytes using the procedure described in Examples G and J.

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Preferred Compounds

The compounds of the invention are substituted 6-membered cyclic 1,3 propane prodrugs of certain phosph(on)ates as represented by Formula I:



I

wherein:

V, W, and W' are independently selected from the group consisting of -H, alkyl, aralkyl, alicyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, and 1-alkynyl; or

together V and Z are connected via an additional 3-5 atoms to form a cyclic group containing 5-7 ring atoms, optionally 1 heteroatom, substituted with hydroxy, acyloxy, alkoxycarbonyloxy, or aryloxycarbonyloxy attached to a carbon atom that is three atoms from both Y groups attached to the phosphorus; or

together V and Z are connected via an additional 3-5 atoms to form a cyclic group, optionally containing 1 heteroatom, said cyclic group is fused to an aryl group at the beta and gamma position to the Y adjacent to V;

together V and W are connected via an additional 3 carbon atoms to form an optionally substituted cyclic group containing 6 carbon atoms and substituted with one substituent selected from the group consisting of hydroxy, acyloxy, alkoxycarbonyloxy, alkylthiocarbonyloxy, and aryloxycarbonyloxy, attached to one of said additional carbon atoms that is three atoms from a Y attached to the phosphorus;

together Z and W are connected via an additional 3-5 atoms to form a cyclic group, optionally containing one heteroatom, and V must be aryl, substituted aryl, heteroaryl, or substituted heteroaryl;

together W and W' are connected via an additional 2-5 atoms to form a cyclic group, optionally containing 0-2 heteroatoms, and V must be aryl, substituted aryl, heteroaryl, or substituted heteroaryl;

Z is selected from the group consisting of $-\text{CHR}^2\text{OH}$, $-\text{CHR}^2\text{OC}(\text{O})\text{R}^3$, $-\text{CHR}^2\text{OC}(\text{S})\text{R}^3$, $-\text{CHR}^2\text{OC}(\text{S})\text{OR}^3$, $-\text{CHR}^2\text{OC}(\text{O})\text{SR}^3$, $-\text{CHR}^2\text{OCO}_2\text{R}^3$, $-\text{OR}^2$, $-\text{SR}^2$, $-\text{CHR}^2\text{N}_3$, $-\text{CH}_2\text{aryl}$, $-\text{CH}(\text{aryl})\text{OH}$, $-\text{CH}(\text{CH}=\text{CR}^2_2)\text{OH}$, $-\text{CH}(\text{C}\equiv\text{CR}^2)\text{OH}$, $-\text{R}^2$, $-\text{NR}^2_2$, $-\text{OCOR}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{SCOR}^3$, $-\text{SCO}_2\text{R}^3$, $-\text{NHCOR}^2$, $-\text{NHCO}_2\text{R}^3$, $-\text{CH}_2\text{NHaryl}$, $-(\text{CH}_2)_p\text{OR}^{12}$, and $-(\text{CH}_2)_p\text{SR}^{12}$;

p is an integer 2 or 3;

with the provisos that:

- a) V, Z, W, W' are not all -H; and
- b) when Z is $-\text{R}^2$, then at least one of V, W, and W' is not -H, alkyl, aralkyl, or alicyclic;

R^2 is selected from the group consisting of R^3 and -H;

R^3 is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl;

R^6 is selected from the group consisting of -H, and lower alkyl, acyloxyalkyl, alkoxyalkyl, alkoxyalkoxy alkyl and lower acyl;

R^{12} is selected from the group consisting of -H, and lower acyl;

each Y is independently selected from the group consisting of -O-, $-\text{NR}^6$ - with the proviso that at least one Y is $-\text{NR}^6$ -;

M is selected from the group that attached to PO_3^{2-} , $\text{P}_2\text{O}_6^{3-}$, $\text{P}_3\text{O}_9^{4-}$ or

$\text{P}(\text{O})(\text{NHR}^6)\text{O}^-$ is a biologically active agent, but is not an FBPase inhibitor, and is attached to the phosphorus in formula I via a carbon, oxygen, sulfur or nitrogen atom;

with the provisos that:

- 1) M is not $-\text{NH}(\text{lower alkyl})$, $-\text{N}(\text{lower alkyl})_2$, $-\text{NH}(\text{lower alkylhalide})$, $-\text{N}(\text{lower alkylhalide})_2$, or $-\text{N}(\text{lower alkyl})(\text{lower alkylhalide})$; and
 - 2) R^6 is not lower alkylhalide;
- and pharmaceutically acceptable prodrugs and salts thereof.

In general, preferred substituents, V, Z, W, and W' of formula I are chosen such that they exhibit one or more of the following properties:

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(2) enhance stability in aqueous solution and in the presence of other non-P450 enzymes;

(4) promote the β -elimination reaction following the initial oxidation by producing ring-opened products that have one or more of the following properties:

15 e) limit metabolism of reaction intermediates (e.g. ring-opened ketone);
(5) lead to a non-toxic and non-mutagenic by-product with one or more of the following characteristics. Both properties can be minimized by using substituents that limit Michael additions, *e.g.*:

f) groups that enhance detoxification of the by-product by one or more of the following characteristics:

(6) capable of generating a pharmacologically active product.

Suitable alkyl groups include groups having from 1 to about 20 carbon atoms. Suitable aryl groups include groups having from 1 to about 20 carbon atoms. Suitable aralkyl groups include groups having from 2 to about 21 carbon atoms. Suitable acyloxy groups include groups having from 1 to about 20 carbon atoms. Suitable alkylene groups include groups having from 1 to about 20 carbon atoms. Suitable alicyclic groups include groups having 3 to about 20 carbon atoms. Suitable heteroaryl groups include groups having from 1 to about 20 carbon atoms and from 1 to 4 heteroatoms, preferably independently selected from nitrogen, oxygen, and sulfur. Suitable heteroalicyclic groups include groups having from 2 to about twenty carbon atoms and from 1 to 5 heteroatoms, preferably independently selected from nitrogen, oxygen, phosphorous, and sulfur.

In compounds of formula I, preferably one Y is -O- and one Y is -NR⁶-. When only one Y is -NR⁶-, preferably the Y closest to W and W' is -O-. In another aspect, preferably the Y closest to V is -O-.

In another aspect, both Y groups are -NR⁶-. More preferred are compounds wherein one Y is -O-, and V, W, and W' are independently selected from the group consisting of -H, alkyl, aralkyl, alicyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, and 1-alkynyl, or

together V and W are connected via an additional 3 carbon atoms to form an optionally substituted cyclic group containing 6 carbon atoms and substituted with one substituent selected from the group consisting of hydroxy, acyloxy, alkoxycarbonyloxy, alkylthiocarbonyloxy, and aryloxycarbonyloxy, attached to one of said additional carbon atoms that is three atoms from a Y attached to the phosphorus.

More preferred are such compounds where V is selected from the group consisting of aryl, substituted aryl, heteroaryl, and substituted heteroaryl.

More preferred V groups of formula VI are aryl, substituted aryl, heteroaryl, and substituted heteroaryl. Preferably, one Y is -O-. Particularly preferred aryl and substituted aryl groups include phenyl, and phenyl substituted with 1-3 halogens. Especially preferred are 3,5-dichlorophenyl, 3-bromo-4-fluorophenyl, 3-chlorophenyl, and 3-bromophenyl.

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It is also especially preferred when V is selected from the group consisting of monocyclic heteroaryl and monocyclic substituted heteroaryl containing at least one nitrogen atom. Most preferred is when such heteroaryl and substituted heteroaryl is 4-pyridyl, and 3-bromopyridyl, respectively.

5 In another particularly preferred aspect, one Y group is -O-, Z, W, and W' are H, and V is phenyl substituted with 1-3 halogens. Especially preferred are 3,5-dichlorophenyl, 3-bromo-4-fluorophenyl, 3-chlorophenyl, and 3-bromophenyl.

It is also especially preferred when V is selected from the group consisting of heteroaryl and substituted heteroaryl.

10 Most preferred is when such heteroaryl is 4-pyridyl.

In another aspect, it is preferred when together V and W are connected via an additional 3 carbon atoms to form an optionally substituted cyclic group containing 6 carbon atoms and monosubstituted with one substituent selected from the group consisting of hydroxy, acyloxy, alkoxycarbonyloxy, alkylthiocarbonyloxy, and aryloxy carbonyloxy
15 attached to one of said additional carbon atoms that is three atoms from a Y attached to the phosphorus. In such compounds, it is more preferred when together V and W form a cyclic group selected from the group consisting of $-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-$, $-\text{CH}_2\text{CH}(\text{OCOR}^3)-\text{CH}_2-$, and $-\text{CH}_2\text{CH}(\text{OCO}_2\text{R}^3)-\text{CH}_2-$.

Another preferred V group is 1-alkene. Oxidation by P450 enzymes is known to
20 occur at benzylic and allylic carbons.

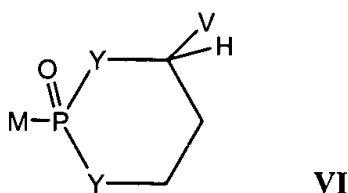
In one aspect, preferred V groups include -H, when Z is $-\text{CHR}^2\text{OH}$, $-\text{CH}_2\text{OCOR}^3$, or $-\text{CH}_2\text{OCO}_2\text{R}^3$.

In another aspect, when V is aryl, substituted aryl, heteroaryl, or substituted heteroaryl, preferred Z groups include $-\text{OR}^2$, $-\text{SR}^2$, $-\text{R}^2$, $-\text{NR}^2$, $-\text{OCOR}^3$, $-\text{OCO}_2\text{R}^3$,
25 $-\text{SCOR}^3$, $-\text{SCO}_2\text{R}^3$, $-\text{NHCOR}^2$, $-\text{NHCO}_2\text{R}^3$, $-(\text{CH}_2)_p-\text{OR}^{12}$, and $-(\text{CH}_2)_p-\text{SR}^{12}$. More preferred Z groups include $-\text{OR}^2$, $-\text{R}^2$, $-\text{OCOR}^2$, $-\text{OCO}_2\text{R}^3$, $-\text{NHCOR}^2$, $-\text{NHCO}_2\text{R}^3$, $-(\text{CH}_2)_p-\text{OR}^{12}$, and $-(\text{CH}_2)_p-\text{SR}^{12}$. Most preferred Z groups include $-\text{OR}^2$, -H, $-\text{OCOR}^3$, $-\text{OCO}_2\text{R}^3$, and $-\text{NHCOR}^2$.

Preferred W and W' groups include H, R^3 , aryl, substituted aryl, heteroaryl, and
30 substituted aryl. Preferably, W and W' are the same group. More preferred is when W and W' are H.

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In one aspect, prodrugs of formula VI which, in addition, are preferred:



wherein

V is selected from the group consisting of aryl, substituted aryl, heteroaryl, and substituted heteroaryl. More preferred are such compounds where M is attached to the phosphorus via an O or N atom. Especially preferred are 3,5-dichlorophenyl, 3-bromo-4-fluorophenyl, 3-chlorophenyl, 3-bromophenyl. Preferably Y is -O-. Particularly preferred aryl and substituted aryl groups include phenyl and substituted phenyl. Particularly preferred heteroaryl groups include monocyclic substituted and unsubstituted heteroaryl groups. Especially preferred are 4-pyridyl and 3-bromopyridyl.

In one aspect, the compounds of formula VI preferably have a group Z which is H, alkyl, alicyclic, hydroxy, alkoxy, OC(O)R^3 , OC(O)OR^3 , or NHC(O)R^2 . Preferred are such groups in which Z decreases the propensity of the by-product, vinylaryl ketone to undergo Michael additions. Preferred Z groups are groups that donate electrons to the vinyl group which is a known strategy for decreasing the propensity of α,β -unsaturated carbonyl compounds to undergo a Michael addition. For example, a methyl group in a similar position on acrylamide results in no mutagenic activity whereas the unsubstituted vinyl analog is highly mutagenic. Other groups could serve a similar function, e.g. $\text{Z}=\text{OR}^{12}$, NHAc, etc. Other groups may also prevent the Michael addition especially groups that result in removal of the double bond altogether such as $\text{Z} = -\text{OH}$, $-\text{OC(O)R}^3$, $-\text{OCO}_2\text{R}^3$, and NH_2 , which will rapidly undergo retautomerization after the elimination reaction. Certain W and W' groups are also advantageous in this role since the group(s) impede the addition reaction to the β -carbon or destabilize the product. Another preferred Z group is one that contains a nucleophilic group capable of adding to the α,β -unsaturated double bond after the elimination reaction *i.e.* $(\text{CH}_2)_p\text{SH}$ or $(\text{CH}_2)_p\text{OH}$ where p is 2 or 3. Yet

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VI

VII

Z is selected from the group consisting of:

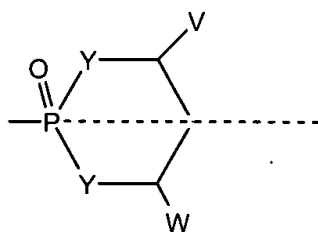
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VIII

wherein

20 D⁴ and D³ are independently selected from the group consisting of -H, alkyl, -OH, and -OC(O)R³; with the proviso that at least one of D⁴ and D³ are -H. Preferably Y is -O-. An especially preferred Z' group is OH.

In one preferred embodiment, W' and Z are -H, W and V are both the same aryl, substituted aryl, heteroaryl, or substituted heteroaryl, and both Y groups are the same - NR⁶-, such that the phosphonate prodrug moiety:

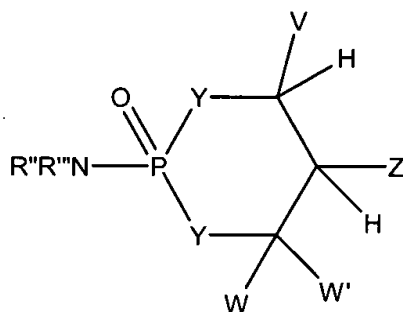


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has a plane of symmetry through the phosphorous-oxygen double bond.

In another preferred embodiment, W and W' are H, V is selected from the group consisting of aryl, substituted aryl, heteroaryl, substituted heteroaryl, and Z is selected from the group consisting of -H, OR², and -NHCOR². More preferred are such compounds where Z is -H. Preferably, such compound have M attached via oxygen. Most preferred are such compounds where oxygen is in a primary hydroxyl group. Also more preferred, are those compounds where V is phenyl or substituted phenyl.

In one aspect, tumor cells expressing a P450 enzyme can be treated by administering a cyclophosphamide analog selected from the group consisting of



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wherein:

V, W, and W' are independently selected from the group consisting of -H, alkyl, aralkyl, alicyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, and 1-alkynyl; or

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5 together V and Z are connected via an additional 3-5 atoms to form a cyclic group, optionally containing 1 heteroatom, said cyclic group is fused to an aryl group at the beta and gamma position to the Y adjacent to V;

together Z and W are connected via an additional 3-5 atoms to form a cyclic group,
optionally containing one heteroatom, and V must be aryl, substituted aryl, heteroaryl, or
15 substituted heteroaryl;

together W and W' are connected via an additional 2-5 atoms to form a cyclic group, optionally containing 0-2 heteroatoms, and V must be aryl, substituted aryl, heteroaryl, or substituted heteroaryl;

Z is selected from the group consisting of $-\text{CHR}^2\text{OH}$, $-\text{CHR}^2\text{OC}(\text{O})\text{R}^3$, $-\text{CHR}^2\text{OC}(\text{S})\text{R}^3$, $-\text{CHR}^2\text{OC}(\text{S})\text{OR}^3$, $-\text{CHR}^2\text{OC}(\text{O})\text{SR}^3$, $-\text{CHR}^2\text{OCO}_2\text{R}^3$, $-\text{OR}^2$, $-\text{SR}^2$, $-\text{CHR}^2\text{N}_3$, $-\text{CH}_2\text{aryl}$, $-\text{CH}(\text{aryl})\text{OH}$, $-\text{CH}(\text{CH}=\text{CR}^2_2)\text{OH}$, $-\text{CH}(\text{C}\equiv\text{CR}^2)\text{OH}$, $-\text{R}^2$, $-\text{NR}^2_2$, $-\text{OCOR}^3$, $-\text{OCO}_2\text{R}^3$, $-\text{SCOR}^3$, $-\text{SCO}_2\text{R}^3$, $-\text{NHCOR}^2$, $-\text{NHCO}_2\text{R}^3$, $-\text{CH}_2\text{NHaryl}$, $-(\text{CH}_2)_p\text{OR}^{12}$, and $-(\text{CH}_2)_n\text{SR}^{12}$;

p is an integer 2 or 3;

25 with the provisos that:

- a) V, Z, W, W' are not all -H; and
- b) when Z is -R², then at least one of V, W, and W' is not -H, alkyl, aralkyl, or alicyclic;

R^2 is selected from the group consisting of R^3 and $-H$;

30 R³ is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl;

R⁶⁶ is selected from the group consisting of -H, lower 2-haloalkyl, and lower alkyl;

R¹² is selected from the group consisting of -H, and lower acyl;

R'' is lower 2-haloalkyl;

R''' is selected from the group consisting of H, lower alkyl, and R'';

each Y is independently selected from the group consisting of -O-, -NR⁶⁶- with the proviso that at least one Y is -NR⁶⁶-;

and pharmaceutically acceptable prodrugs and salts thereof.

5 Preferably, the tumor cell is a hepatocellular carcinoma. These cyclophosphamide analogs can also be used to treat tumor cells by enhancing the activity of a P450 enzyme that oxidizes cyclophosphamide analogs and by administering the cyclophosphamide analogs.

10 In another aspect, the P450 activity is enhanced by the administration of a compound that increases the amount of endogenous P450 enzyme. Preferably, the compound that increases the amount of endogenous P450 enzyme is selected from the group consisting of phenobarbital, dexamethasone, rifampicin, phenytoin, and pregnanolon-16 α -carbonitrile.

15 Preferred cyclophosphoramide analogs include those where R'' is 2-chloroethyl, and R''' is selected from the group consisting of -H, and 2-chloroethyl.

Also, preferred are cyclophosphamide analogs where R⁶⁶ is selected from the group consisting of -H, methyl, and 2-chloroethyl.

In one aspect, the activity of a P450 enzyme is enhanced by administration of a compound that increases the amount of endogenous P450 enzyme.

20 Also preferred cyclophosphamide analogs are those where Z, W, W', and R⁶⁶ are -H, and R'' and R''' are 2-chloroethyl.

25 Preferred cyclophosphamide analogs have V is selected from the group consisting of aryl, substituted aryl, heteroaryl, and substituted heteroaryl. More preferred are those compounds where Z, W, W', and R⁶⁶ are -H, and R'' and R''' are 2-chloroethyl. Also more preferred are those wherein V is selected from the group consisting of phenyl, 3-chlorophenyl, and 3-bromophenyl. Also preferred are those wherein V is 4-pyridyl.

30 Also more preferred, are those compounds where V is an optionally substituted monocyclic heteroaryl containing at least one nitrogen atom. Preferably such compounds have M attached via oxygen. Most preferred are such compounds where said oxygen is in a primary hydroxyl group. Especially preferred are such compounds where V is 4-pyridyl.

Preferably, oral bioavailability is at least 5%. More preferably, oral bioavailability is at least 10%.

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P450 oxidation can be sensitive to stereochemistry which might either be at phosphorus or at the carbon bearing the aromatic group. The prodrugs of the present invention have two isomeric forms around the phosphorus. Preferred is the stereochemistry that enables both oxidation and the elimination reaction. Preferred is the cis-stereochemistry. In contrast, the reaction is relatively insensitive to the group M since cleavage occurred with a variety of phosphonate, phosphate and phosphoramidates. The atom in M attached to phosphorus may be O, S or N. The active drug is $M-PO_3^{2-}$, $MP_2O_6^{3-}$, or $MP_3O_9^{4-}$ useful for treatment of diseases in which the liver is a target organ, including diabetes, hepatitis, liver cancer, liver fibrosis, malaria and metabolic diseases where the liver is responsible for the overproduction of a biochemical end products such as glucose (diabetes), cholesterol, fatty acids and triglycerides (atherosclerosis). Moreover, $M-PO_3^{2-}$, $MP_2O_6^{3-}$, or $MP_3O_9^{4-}$ may be useful in treating diseases where the target is outside the liver in tissues or cells that can oxidize the prodrug.

Other preferred M groups include drugs useful in treating diabetes, cancer, viral infections, liver fibrosis, parasitic infections, hypertension, and hyperlipidemia.

The preferred compounds of formula VIII utilize a Z' group that is capable of undergoing an oxidative reaction that yields an unstable intermediate which via elimination reactions breaks down to the corresponding $M-P(O)(NHR^6)_2$, or $M-P(O)(O^-)(NHR^6)$. An especially preferred Z' group is OH. Groups D^4 and D^3 are preferably hydrogen, alkyl, $-OR^2$, $-OCOR^3$, but at least one of D^4 or D^3 must be H.

The following compounds and their analogs can be used in the prodrug methodology of the present invention.

In one preferred aspect, M is attached to the phosphorus in formula I via an oxygen atom. Preferably, M is a nucleoside. Preferably, M is attached via an oxygen that is in a primary hydroxyl group on a ribofuranosyl or an arabinofuranosyl group. Preferably such compounds include LdT, LdC, araA; AZT; d4T; ddI; ddA; ddC; L-ddC; L FddC; L-d4C; L-Fd4C; 3TC; ribavirin; 5-fluoro 2'deoxyuridine; FIAU; FIAC; BHCG; L FMAU; BvaraU; E-5-(2-bromovinyl-2' deoxyuridine; TFT; 5-propynyl-1 arabinosyluracil; CDG; DAPD; FDOC; d4C; DXG; FEAU; FLG; FLT; FTC; 5-yl-carbocyclic 2'deoxyguanosine; oxetanocin A; oxetanocin G; Cyclobut A, Cyclobut G; dFdC; araC; bromodeoxyuridine; IDU; CdA; FaraA; Coformycin, 2'-deoxycoformycin; araT; tiazofurin; ddAPR; 9-(arabinofuranosyl)-2,6 diaminopurine; 9-(2'-deoxyribofuranosyl)-2,6 diaminopurine; 9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine; 9 (arabinofuranosyl)guanine; 9-(2'

deoxyribofuranosyl)guanine; 9-(2'-deoxy 2'fluororibofuranosyl)guanine; FMdC; 5,6 dihydro-5-azacytidine; 5-azacytidine; 5-aza 2'deoxycytidine; or AICAR. In another aspect, it is preferred when M is attached via an oxygen in a hydroxyl on an acyclic sugar it is preferred when such MH is ACV, GCV; penciclovir; (R)-9-(3,4

5 dihydroxybutyl)guanine; or cytallene.

In one preferred aspect, the compounds in formula I do not include compounds where MH is araA or 5-fluoro-2'-deoxyuridine.

In general, it is preferred that when M is attached via an oxygen, said oxygen is in a primary hydroxy group. In such an instance, it is preferred that MH is araA; AZT; d4T; 10 ddI; ddA; ddC; L-ddC; L FddC; L-d4C; L-Fd4C; 3TC; ribavirin; 5-fluoro 2'deoxyuridine; FIAU; FIAC; BHCG; L FMAU; BvaraU; E-5-(2-bromovinyl-2' deoxyuridine; TFT; 5-propynyl-1 arabinosyluracil; CDG; DAPD; FDOC; d4C; DXG; FEAU; FLG; FLT; FTC; 5-yl-carbocyclic 2'deoxyguanosine; oxetanocin A; oxetanocin G; Cyclobut A, Cyclobut G; dFdC; araC; bromodeoxyuridine; IDU; CdA; FaraA; Coformycin, 2'-deoxycostamycin; 15 araT; tiazofurin; ddAPR; 9-(arabinofuranosyl)-2,6 diaminopurine; 9-(2'-deoxyribofuranosyl)-2,6 diaminopurine; 9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine; 9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine; 9-(2'-deoxy 2'fluororibofuranosyl)guanine; FMdC; 5,6 dihydro-5-azacytidine; 5-azacytidine; 5-aza 2'deoxycytidine; AICAR; ACV, GCV; penciclovir; (R)-9-(3,4 20 dihydroxybutyl)guanine; or cytallene.

In another aspect, MH is araA.

In another aspect, MH is 5-fluoro-2'-deoxyuridine.

In another aspect, compounds of formula I wherein M is attached to the phosphorus in formula I via a carbon atom are preferred. In such compounds, preferably 25 $M-PO_3^{2-}$ is phosphonoformic acid, or phosphonoacetic acid.

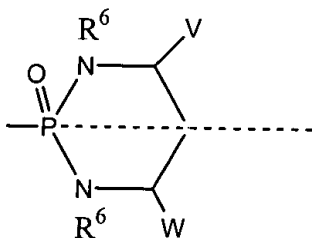
In another preferred aspect, MPO_3^{2-} , $MP_2O_6^{3-}$, or $MP_3O_9^{4-}$ is useful for the treatment of diseases of the liver or metabolic diseases where the liver is responsible for the overproduction of a biochemical end product. Preferably, such disease of the liver is selected from the group consisting of diabetes, hepatitis, cancer, fibrosis, malaria, 30 gallstones, and chronic cholecystolithiasis. It is more preferred when treating such diseases that MH, MPO_3^{2-} , $MP_2O_6^{3-}$, or $MP_3O_9^{4-}$ is an antiviral or anticancer agent.

Preferably, the metabolic disease that MPO_3^{2-} , $MP_2O_6^{3-}$, or $MP_3O_9^{4-}$ are useful for diabetes, atherosclerosis, and obesity.

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In another aspect, it is preferred when the biochemical end product is selected from the group consisting of glucose, cholesterol, fatty acids, and triglycerides. More preferred is when $\text{MP}(\text{O})(\text{NHR}^6)\text{O}^-$ or MPO_3^{2-} is an AMP activated protein kinase activator.

In one preferred embodiment, W' and Z are $-\text{H}$, W and V are both the same aryl, substituted aryl, heteroaryl, or substituted heteroaryl such that the phosphonate prodrug moiety:



has a plane of symmetry.

In another preferred embodiment, W and W' are H , V is selected from the group consisting of aryl, substituted aryl, heteroaryl, substituted heteroaryl, and Z is selected from the group consisting of $-\text{H}$, OR^2 , and $-\text{NHCOR}^2$. More preferred are such compounds where Z is $-\text{H}$. Preferably, such compound have M attached via oxygen. Most preferred are such compounds where oxygen is in a primary hydroxyl group.

Also more preferred, are those compounds where V is phenyl or substituted phenyl. Most preferred are such compounds where said oxygen is in a primary hydroxyl group.

Preferably, such compounds have M attached via oxygen.

Also more preferred, are those compounds where V is an optionally substituted monocyclic heteroaryl containing at least one nitrogen atom. Preferably such compounds have M attached via oxygen. Most preferred are such compounds where said oxygen is in a primary hydroxyl group.

Especially preferred are such compounds where V is selected from the group consisting of phenyl substituted with 1-3 halogens, and 4-pyridyl. In these compounds it is also preferred when MH is selected from the group consisting of LdT , LdC , araA ; AZT ; d4T ; ddI ; ddA ; ddC ; L-ddC ; L-FddC ; L-d4C ; L-Fd4C ; 3TC ; ribavirin; 5-fluoro 2'deoxyuridine; FIAU ; FIAC ; BHCG ; L-FMAU ; BvaraU ; $\text{E-5-(2-bromovinyl-2'}$ deoxyuridine; TFT ; 5-propynyl-1 arabinosyluracil; CDG ; DAPD ; FDOC ; d4C ; DXG ; FEAU ; FLG ; FLT ; FTC ; 5-yl-carbocyclic 2'deoxyguanosine; oxetanocin A; oxetanocin G;

Cyclobut A, Cyclobut G; dFdC; araC; bromodeoxyuridine; IDU; CdA; FaraA; Coformycin, 2'-deoxycoformycin; araT; tiazofurin; ddAPR; 9-(arabinofuranosyl)-2,6 diaminopurine; 9-(2'-deoxyribofuranosyl)-2,6 diaminopurine; 9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine; 9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine; 9-(2'-deoxy 2'fluororibofuranosyl)guanine; FMdC; 5,6 dihydro-5-azacytidine; 5-azacytidine; 5-aza 2'deoxyctidine; AICAR; ACV, GCV; penciclovir; (R)-9-(3,4 dihydroxybutyl)guanine; or cyttallene. Particularly preferred are such compounds where V is selected from the group consisting of phenyl substituted with 1-3 halogens and 4-pyridyl and MH is selected from the group consisting of araA; AZT; d4T; 3TC; ribavirin; 5 fluoro-2'deoxyuridine; FMAU; DAPD; FTC; 5-yl-carbocyclic 2'deoxyguanosine; Cyclobut G; dFdC; araC; IDU; FaraA; ACV; GCV; or penciclovir.

Also preferred is when MH is selected from the group consisting of ACV, GCV; penciclovir; (R)-9-(3,4 dihydroxybutyl)guanine; cyttallene.

When W' and W are H, V is aryl, substituted aryl, heteroaryl, or substituted heteroaryl, and Z is H, OR², or -NHCOR², it is also preferred when M is attached to the phosphorus via a carbon atom. Preferred are such compounds wherein MPO₃²⁻ is selected from the group consisting of phosphonoformic acid, and phosphonoacetic acid. Also preferred are MH is selected from the group consisting of PMEA, PMEDAP, HPMPC, HPMPA, FPMMPA, and PMPA.

Preferably, oral bioavailability is at least 5%. More preferably, oral bioavailability is at least 10%.

P450 oxidation can be sensitive to stereochemistry which might either be at phosphorus or at the carbon bearing the aromatic group. The prodrugs of the present invention have two isomeric forms around the phosphorus. Preferred is the stereochemistry that enables both oxidation and the elimination reaction. Preferred is the cis stereochemistry. In contrast, the reaction is relatively insensitive to the group M since cleavage occurred with a variety of phosphonate, phosphate and phosphoramidates. Accordingly, the group M represents a group that as part of a compound of formula I enables generation of a biologically active compound in vivo via conversion to the corresponding M-PO₃²⁻. The atom in M attached to phosphorus may be O, S, C or N. The active drug may be M-PO₃²⁻ or a metabolite of M-PO₃²⁻ such as the triphosphate useful for treatment of diseases in which the liver is a target organ, including diabetes, hepatitis, liver cancer, liver fibrosis, malaria and metabolic diseases where the liver is responsible for the

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overproduction of a biochemical end products such as glucose (diabetes), cholesterol, fatty acids and triglycerides (atherosclerosis). Moreover, $M-PO_3^{2-}$ and $MP(O)(NHR^6)O^-$ may be useful in treating diseases where the target is outside the liver but accessible to a phosph(on)ate. Preferred M groups are groups in which M is a nucleoside and the

5 phosphate is attached to a hydroxyl, preferably a primary hydroxyl on a sugar or sugar-analog. More preferred M groups include LdC, LdT, araA; AZT; d4T; ddI; ddA; ddC; L-ddC; L FddC; L-d4C; L-Fd4C; 3TC; ribavirin; 5-fluoro 2'deoxyuridine; FIAU; FIAC; BHCG; L FMAU; BvaraU; E-5-(2-bromovinyl-2' deoxyuridine; TFT; 5-propynyl-1 arabinosyluracil; CDG; DAPD; FDOC; d4C; DXG; FEAU; FLG; FLT; FTC; 5-yl-
10 carbocyclic 2'deoxyguanosine; oxetanocin A; oxetanocin G; Cyclobut A, Cyclobut G; dFdC; araC; bromodeoxyuridine; IDU; CdA; FaraA; Coformycin, 2'-deoxycoformycin; araT; tiazofurin; ddAPR; 9-(arabinofuranosyl)-2,6 diaminopurine; 9-(2'-deoxyribofuranosyl)-2,6 diaminopurine; 9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine; 9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine; 9-(2'-
15 deoxy 2'fluororibofuranosyl)guanine; FMdC; 5,6 dihydro-5-azacytidine; 5-azacytidine; 5-aza 2'deoxycytidine; AICAR; ACV, GCV; penciclovir; (R)-9-(3,4 dihydroxybutyl)guanine; or cytallene. Especially preferred are lobucavir, FTC, 3TC, BMS 200,475; DAPD, DXG, L-FMAU, LdC, LdT, ribavirin, F-ara-A, araC, CdA, dFdC, 5-fluoro-2'-deoxyuridine, ACV, GCV, and penciclovir.

20 The preferred M groups include phosphonic acids useful for treating viral infections. Such preferred antivirals include PMEA; PMEDAP; HPMPC; HPMPA; FPMPPA; PMPA; foscarnet; phosphonoformic acid. More preferred are PMEA, PMPA, HPMPC, and HPMPA. Especially preferred are PMEA and PMPA.

Other preferred M groups include phosphonic acids useful in treating diabetes,
25 liver fibrosis, e.g. collagenase inhibitors such as reported in Bird *et al.*, *J. Med. Chem.* **37**, 158-169 (1994), parasitic infections, diseases responsive to metalloprotease inhibition (e.g. hypertension, liver, cancer), and hyperlipidemia.

In another aspect, preferred MH groups including L-nucleosides. Preferred L-nucleosides include FTC, 3TC, L-FMAU, LdC, and LdT.

30 In one aspect, preferred MH groups are acyclic nucleosides. Preferred acyclic nucleosides include ACV, GCV; penciclovir; (R)-9-(3,4 dihydroxybutyl)guanine; and cytallene. More preferred are ACV; GCV, and penciclovir.

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In another aspect, preferred MH groups include dideoxy nucleosides. Preferred dideoxy nucleosides include AZT; d4T; ddI; ddA; ddC; L-ddC; L-FddC; L d4C; L-Fd4C; d4C; and ddAPR. More preferred are AZT; d4T; ddI; and ddC.

In another aspect, preferred MH groups include arabinofuranosyl nucleosides.
5 Preferred are araA; araT; 5-propynyl-1-arabinosyluracil; araC; FaraA; 9-(arabinofuranosyl)-2,6 diaminopurine; and 9-(arabinofuranosyl)guanine. More preferred are araA; araC; and FaraA.

In another aspect, preferred MH groups include carbocyclic nucleosides. Preferred are 5-yl-carbocyclic 2'deoxyguanosine; CDG; cyclobut A; cyclobut G; and BHCG. More
10 preferred are 5-yl-carbocyclic 2'deoxyguanosine; and cyclobut G.

In another aspect, preferred MH groups include fluorinated sugars on the nucleosides. Preferred fluorinated sugars include FLT; FLG; FIAC; FIAU; FMAU; FEAU; dFdC; 9-(2'-deoxy-2'fluororibofuranosyl) 2,6-diaminopurine; and 9-(2'-deoxy 2'fluororibofuranosyl)guanine. More preferred are L-FMAU; and dFdC.

15 In another aspect, preferred MH groups include dioxolane nucleosides. Preferred dioxolane nucleosides include DAPD; DXG; and FDOC. More preferred is DAPD and DXG.

In another aspect, preferred MH and MPO_3^{2-} for treating viral infections include lobucavir, FTC, 3TC, BMS 200,475; DAPD, DXG, L-FMAU, LdC, LdT, ribavirin, ACV,
20 GCV, penciclovir, PMEAs, and PMPA.

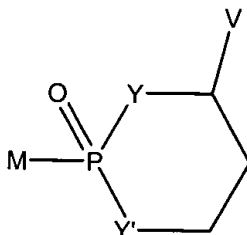
In another aspect, preferred MH groups for treating cancer include F-ara-A, araC, CdA, dFdc, and 5-fluoro-2'-deoxyuridine.

The following prodrugs are preferred compounds of the invention. The compounds are shown without depiction of stereochemistry since the compounds are
25 biologically active as the diastereomeric mixture or as a single stereoisomer. Compounds named in Table 1 are designated by numbers assigned to the variables of formula VIa using the following convention: $\text{M}^1\text{.V.L1.L2}$ where Y is NH and Y' is oxygen; M^1 is a variable that represents compounds of the formula M-H which have a specific hydroxyl group that is phosphorylated with a group $\text{P}(\text{O})(\text{Y}-\text{CH}(\text{V})\text{CH}_2\text{CH}_2-\text{Y}')$ to make
30 compounds of formula VIa or M^1 is a variable that represents phosphonic acids of the formula $\text{M}-\text{PO}_3^{2-}$ which are transformed to compounds of formula VIa by replacing two

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oxygen in the PO_3^{2-} group with $\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'}$. V is an aryl or heteroaryl group that has 2 substituents, L1 and L2, at the designated positions.

Variable M^1 is divided into three groups with the structures assigned to each group listed below:



VI a

5

Variable M^1 : Group M^1 1:

- 1) 3TC where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl.
- 2) (-)FTC where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl.
- 3) L-FMAU where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl.
- 4) Penciclovir where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl that is phosphorylated in cells.
- 5) BMS 200,475 where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl.
- 6) L(-)Fd4C where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl.
- 7) Lobucavir where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl that is phosphorylated in cells.
- 8) DXG where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl.
- 9) LdC where $-\text{P(O)}(\text{Y-CH(V)CH}_2\text{CH}_2\text{-Y'})$ is attached to the primary hydroxyl.

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Variable M¹: Group M¹2:

- 1) ddI where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 5 2) LdT where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 3) ddC where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 10 4) AZT where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 5) d4T where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 6) DAPD where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 15 7) L-FddC where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 8) Ribavirin where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 20 9) CdA where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl

Variable M¹: Group M¹3:

- 25 1) Ganciclovir where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl that is phosphorylated in cells.
- 2) Acyclovir where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 3) Cytarabine where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl.
- 30 4) Gemcitabine where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl
- 5) Fludarabine where -P(O)(Y-CH(V)CH₂CH₂-Y') is attached to the primary hydroxyl

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- 10

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- 20

25

- 30

Variable V: Group V3

- | | | |
|----|----|------------------------|
| | 1) | 4-(L1)-6(L2) 3-pyridyl |
| | 2) | 5-(L1)-6(L2) 3-pyridyl |
| 5 | 3) | 3-(L1)-4(L2) 2-pyridyl |
| | 4) | 3-(L1)-5(L2) 2-pyridyl |
| | 5) | 3-(L1)-6(L2) 2-pyridyl |
| | 6) | 4-(L1)-5(L2) 2-pyridyl |
| | 7) | 4-(L1)-6(L2) 2-pyridyl |
| 10 | 8) | 3-(L1)-4(L2)-2-thienyl |
| | 9) | 2-(L1)-5(L2) 3-furanyl |

Variable L1

- | | | |
|----|----|---------------|
| | 1) | hydrogen |
| 15 | 2) | chloro |
| | 3) | bromo |
| | 4) | fluoro |
| | 5) | methyl |
| | 6) | isopropyl |
| 20 | 7) | methoxy |
| | 8) | dimethylamino |
| | 9) | acyloxy |

Variable L2

- | | | |
|----|----|---------------|
| | 1) | hydrogen |
| 25 | 2) | chloro |
| | 3) | bromo |
| | 4) | fluoro |
| | 5) | methyl |
| | 6) | isopropyl |
| 30 | 7) | methoxy |
| | 8) | dimethylamino |
| | 9) | acyloxy |

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Preferred compounds are compounds listed in Table 1 using groups M¹1 and V1.
For example, compound 1.3.6.7 represents structure 1 of group M¹1, i.e. 3TC; structure 3
of group V1, i.e. 2-(L1)-5-(L2) phenyl; structure 6 of variable L1, i.e. isopropyl; and
structure 7 of variable L2, i.e. methoxy. The compound 1.3.6.7. therefore is 3TC with the
5 P(O)(Y-CH(V)CH₂CH₂Y') attached to the primary hydroxyl group being {[1-(2-*I*-propyl-
5-methoxyphenyl)-1,3-propyl]phosphoryl}.

Preferred compounds are also compounds listed in Table 1 using groups M¹1 and
V2.

Preferred compounds are also compounds listed in Table 1 using groups M¹1 and
10 V3.

Preferred compounds are also compounds listed in Table 1 using groups M¹2 and
V1.

Preferred compounds are also compounds listed in Table 1 using groups M¹2 and
V2.

Preferred compounds are also compounds listed in Table 1 using groups M¹2 and
15 V3.

Preferred compounds are also compounds listed in Table 1 using groups M¹3 and
V1.

Preferred compounds are also compounds listed in Table 1 using groups M¹3 and
20 V2.

Preferred compounds are also compounds listed in Table 1 using groups M¹3 and
V3.

Preferred compounds are represented by all of the above named compounds with
the exception that Y is oxygen and Y' is NH.

Preferred compounds are represented by all of the above named compounds with
25 the exception that Y and Y' are NH.

Preferred compounds are represented by all of the above named compounds with
the exception that Y is NCH₃ and Y' is oxygen.

Preferred compounds are represented by all of the above named compounds with
30 the exception that Y is oxygen and Y' is NCH₃.

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1.1.1.1	1.1.1.2	1.1.1.3	1.1.1.4	1.1.1.5	1.1.1.6	1.1.1.7	1.1.1.8	1.1.1.9	1.1.2.1
1.1.2.2	1.1.2.3	1.1.2.4	1.1.2.5	1.1.2.6	1.1.2.7	1.1.2.8	1.1.2.9	1.1.3.1	1.1.3.2
1.1.3.3	1.1.3.4	1.1.3.5	1.1.3.6	1.1.3.7	1.1.3.8	1.1.3.9	1.1.4.1	1.1.4.2	1.1.4.3
1.1.4.4	1.1.4.5	1.1.4.6	1.1.4.7	1.1.4.8	1.1.4.9	1.1.5.1	1.1.5.2	1.1.5.3	1.1.5.4
1.1.5.5	1.1.5.6	1.1.5.7	1.1.5.8	1.1.5.9	1.1.6.1	1.1.6.2	1.1.6.3	1.1.6.4	1.1.6.5
1.1.6.6	1.1.6.7	1.1.6.8	1.1.6.9	1.1.7.1	1.1.7.2	1.1.7.3	1.1.7.4	1.1.7.5	1.1.7.6
1.1.7.7	1.1.7.8	1.1.7.9	1.1.8.1	1.1.8.2	1.1.8.3	1.1.8.4	1.1.8.5	1.1.8.6	1.1.8.7
1.1.8.8	1.1.8.9	1.1.9.1	1.1.9.2	1.1.9.3	1.1.9.4	1.1.9.5	1.1.9.6	1.1.9.7	1.1.9.8
1.1.9.9	1.2.1.1	1.2.1.2	1.2.1.3	1.2.1.4	1.2.1.5	1.2.1.6	1.2.1.7	1.2.1.8	1.2.1.9
1.2.2.1	1.2.2.2	1.2.2.3	1.2.2.4	1.2.2.5	1.2.2.6	1.2.2.7	1.2.2.8	1.2.2.9	1.2.3.1
1.2.3.2	1.2.3.3	1.2.3.4	1.2.3.5	1.2.3.6	1.2.3.7	1.2.3.8	1.2.3.9	1.2.4.1	1.2.4.2
1.2.4.3	1.2.4.4	1.2.4.5	1.2.4.6	1.2.4.7	1.2.4.8	1.2.4.9	1.2.5.1	1.2.5.2	1.2.5.3
1.2.5.4	1.2.5.5	1.2.5.6	1.2.5.7	1.2.5.8	1.2.5.9	1.2.6.1	1.2.6.2	1.2.6.3	1.2.6.4
1.2.6.5	1.2.6.6	1.2.6.7	1.2.6.8	1.2.6.9	1.2.7.1	1.2.7.2	1.2.7.3	1.2.7.4	1.2.7.5
1.2.7.6	1.2.7.7	1.2.7.8	1.2.7.9	1.2.8.1	1.2.8.2	1.2.8.3	1.2.8.4	1.2.8.5	1.2.8.6
1.2.8.7	1.2.8.8	1.2.8.9	1.2.9.1	1.2.9.2	1.2.9.3	1.2.9.4	1.2.9.5	1.2.9.6	1.2.9.7
1.2.9.8	1.2.9.9	1.3.1.1	1.3.1.2	1.3.1.3	1.3.1.4	1.3.1.5	1.3.1.6	1.3.1.7	1.3.1.8
1.3.1.9	1.3.2.1	1.3.2.2	1.3.2.3	1.3.2.4	1.3.2.5	1.3.2.6	1.3.2.7	1.3.2.8	1.3.2.9
1.3.3.1	1.3.3.2	1.3.3.3	1.3.3.4	1.3.3.5	1.3.3.6	1.3.3.7	1.3.3.8	1.3.3.9	1.3.4.1
1.3.4.2	1.3.4.3	1.3.4.4	1.3.4.5	1.3.4.6	1.3.4.7	1.3.4.8	1.3.4.9	1.3.5.1	1.3.5.2
1.3.5.3	1.3.5.4	1.3.5.5	1.3.5.6	1.3.5.7	1.3.5.8	1.3.5.9	1.3.6.1	1.3.6.2	1.3.6.3
1.3.6.4	1.3.6.5	1.3.6.6	1.3.6.7	1.3.6.8	1.3.6.9	1.3.7.1	1.3.7.2	1.3.7.3	1.3.7.4
1.3.7.5	1.3.7.6	1.3.7.7	1.3.7.8	1.3.7.9	1.3.8.1	1.3.8.2	1.3.8.3	1.3.8.4	1.3.8.5
1.3.8.6	1.3.8.7	1.3.8.8	1.3.8.9	1.3.9.1	1.3.9.2	1.3.9.3	1.3.9.4	1.3.9.5	1.3.9.6
1.3.9.7	1.3.9.8	1.3.9.9	1.4.1.1	1.4.1.2	1.4.1.3	1.4.1.4	1.4.1.5	1.4.1.6	1.4.1.7
1.4.1.8	1.4.1.9	1.4.2.1	1.4.2.2	1.4.2.3	1.4.2.4	1.4.2.5	1.4.2.6	1.4.2.7	1.4.2.8
1.4.2.9	1.4.3.1	1.4.3.2	1.4.3.3	1.4.3.4	1.4.3.5	1.4.3.6	1.4.3.7	1.4.3.8	1.4.3.9
1.4.4.1	1.4.4.2	1.4.4.3	1.4.4.4	1.4.4.5	1.4.4.6	1.4.4.7	1.4.4.8	1.4.4.9	1.4.5.1
1.4.5.2	1.4.5.3	1.4.5.4	1.4.5.5	1.4.5.6	1.4.5.7	1.4.5.8	1.4.5.9	1.4.6.1	1.4.6.2
1.4.6.3	1.4.6.4	1.4.6.5	1.4.6.6	1.4.6.7	1.4.6.8	1.4.6.9	1.4.7.1	1.4.7.2	1.4.7.3
1.4.7.4	1.4.7.5	1.4.7.6	1.4.7.7	1.4.7.8	1.4.7.9	1.4.8.1	1.4.8.2	1.4.8.3	1.4.8.4
1.4.8.5	1.4.8.6	1.4.8.7	1.4.8.8	1.4.8.9	1.4.9.1	1.4.9.2	1.4.9.3	1.4.9.4	1.4.9.5
1.4.9.6	1.4.9.7	1.4.9.8	1.4.9.9	1.5.1.1	1.5.1.2	1.5.1.3	1.5.1.4	1.5.1.5	1.5.1.6

Table 1 - continued

	1.7.3.7	1.7.3.8	1.7.3.9	1.7.4.1	1.7.4.2	1.7.4.3	1.7.4.4	1.7.4.5	1.7.4.6	1.7.4.7
	1.7.4.8	1.7.4.9	1.7.5.1	1.7.5.2	1.7.5.3	1.7.5.4	1.7.5.5	1.7.5.6	1.7.5.7	1.7.5.8
	1.7.5.9	1.7.6.1	1.7.6.2	1.7.6.3	1.7.6.4	1.7.6.5	1.7.6.6	1.7.6.7	1.7.6.8	1.7.6.9
5	1.7.7.1	1.7.7.2	1.7.7.3	1.7.7.4	1.7.7.5	1.7.7.6	1.7.7.7	1.7.7.8	1.7.7.9	1.7.8.1
	1.7.8.2	1.7.8.3	1.7.8.4	1.7.8.5	1.7.8.6	1.7.8.7	1.7.8.8	1.7.8.9	1.7.9.1	1.7.9.2
	1.7.9.3	1.7.9.4	1.7.9.5	1.7.9.6	1.7.9.7	1.7.9.8	1.7.9.9	1.8.1.1	1.8.1.2	1.8.1.3
	1.8.1.4	1.8.1.5	1.8.1.6	1.8.1.7	1.8.1.8	1.8.1.9	1.8.2.1	1.8.2.2	1.8.2.3	1.8.2.4
	1.8.2.5	1.8.2.6	1.8.2.7	1.8.2.8	1.8.2.9	1.8.3.1	1.8.3.2	1.8.3.3	1.8.3.4	1.8.3.5
10	1.8.3.6	1.8.3.7	1.8.3.8	1.8.3.9	1.8.4.1	1.8.4.2	1.8.4.3	1.8.4.4	1.8.4.5	1.8.4.6
	1.8.4.7	1.8.4.8	1.8.4.9	1.8.5.1	1.8.5.2	1.8.5.3	1.8.5.4	1.8.5.5	1.8.5.6	1.8.5.7
	1.8.5.8	1.8.5.9	1.8.6.1	1.8.6.2	1.8.6.3	1.8.6.4	1.8.6.5	1.8.6.6	1.8.6.7	1.8.6.8
	1.8.6.9	1.8.7.1	1.8.7.2	1.8.7.3	1.8.7.4	1.8.7.5	1.8.7.6	1.8.7.7	1.8.7.8	1.8.7.9
	1.8.8.1	1.8.8.2	1.8.8.3	1.8.8.4	1.8.8.5	1.8.8.6	1.8.8.7	1.8.8.8	1.8.8.9	1.8.9.1
15	1.8.9.2	1.8.9.3	1.8.9.4	1.8.9.5	1.8.9.6	1.8.9.7	1.8.9.8	1.8.9.9	1.9.1.1	1.9.1.2
	1.9.1.3	1.9.1.4	1.9.1.5	1.9.1.6	1.9.1.7	1.9.1.8	1.9.1.9	1.9.2.1	1.9.2.2	1.9.2.3
	1.9.2.4	1.9.2.5	1.9.2.6	1.9.2.7	1.9.2.8	1.9.2.9	1.9.3.1	1.9.3.2	1.9.3.3	1.9.3.4
	1.9.3.5	1.9.3.6	1.9.3.7	1.9.3.8	1.9.3.9	1.9.4.1	1.9.4.2	1.9.4.3	1.9.4.4	1.9.4.5
	1.9.4.6	1.9.4.7	1.9.4.8	1.9.4.9	1.9.5.1	1.9.5.2	1.9.5.3	1.9.5.4	1.9.5.5	1.9.5.6
20	1.9.5.7	1.9.5.8	1.9.5.9	1.9.6.1	1.9.6.2	1.9.6.3	1.9.6.4	1.9.6.5	1.9.6.6	1.9.6.7
	1.9.6.8	1.9.6.9	1.9.7.1	1.9.7.2	1.9.7.3	1.9.7.4	1.9.7.5	1.9.7.6	1.9.7.7	1.9.7.8
	1.9.7.9	1.9.8.1	1.9.8.2	1.9.8.3	1.9.8.4	1.9.8.5	1.9.8.6	1.9.8.7	1.9.8.8	1.9.8.9
	1.9.9.1	1.9.9.2	1.9.9.3	1.9.9.4	1.9.9.5	1.9.9.6	1.9.9.7	1.9.9.8	1.9.9.9	2.1.1.1
	2.1.1.2	2.1.1.3	2.1.1.4	2.1.1.5	2.1.1.6	2.1.1.7	2.1.1.8	2.1.1.9	2.1.2.1	2.1.2.2
25	2.1.2.3	2.1.2.4	2.1.2.5	2.1.2.6	2.1.2.7	2.1.2.8	2.1.2.9	2.1.3.1	2.1.3.2	2.1.3.3
	2.1.3.4	2.1.3.5	2.1.3.6	2.1.3.7	2.1.3.8	2.1.3.9	2.1.4.1	2.1.4.2	2.1.4.3	2.1.4.4
	2.1.4.5	2.1.4.6	2.1.4.7	2.1.4.8	2.1.4.9	2.1.5.1	2.1.5.2	2.1.5.3	2.1.5.4	2.1.5.5
	2.1.5.6	2.1.5.7	2.1.5.8	2.1.5.9	2.1.6.1	2.1.6.2	2.1.6.3	2.1.6.4	2.1.6.5	2.1.6.6
	2.1.6.7	2.1.6.8	2.1.6.9	2.1.7.1	2.1.7.2	2.1.7.3	2.1.7.4	2.1.7.5	2.1.7.6	2.1.7.7
30	2.1.7.8	2.1.7.9	2.1.8.1	2.1.8.2	2.1.8.3	2.1.8.4	2.1.8.5	2.1.8.6	2.1.8.7	2.1.8.8
	2.1.8.9	2.1.9.1	2.1.9.2	2.1.9.3	2.1.9.4	2.1.9.5	2.1.9.6	2.1.9.7	2.1.9.8	2.1.9.9
	2.2.1.1	2.2.1.2	2.2.1.3	2.2.1.4	2.2.1.5	2.2.1.6	2.2.1.7	2.2.1.8	2.2.1.9	2.2.2.1
	2.2.2.2	2.2.2.3	2.2.2.4	2.2.2.5	2.2.2.6	2.2.2.7	2.2.2.8	2.2.2.9	2.2.3.1	2.2.3.2
	2.2.3.3	2.2.3.4	2.2.3.5	2.2.3.6	2.2.3.7	2.2.3.8	2.2.3.9	2.2.4.1	2.2.4.2	2.2.4.3
35	2.2.4.4	2.2.4.5	2.2.4.6	2.2.4.7	2.2.4.8	2.2.4.9	2.2.5.1	2.2.5.2	2.2.5.3	2.2.5.4
	2.2.5.5	2.2.5.6	2.2.5.7	2.2.5.8	2.2.5.9	2.2.6.1	2.2.6.2	2.2.6.3	2.2.6.4	2.2.6.5
	2.2.6.6	2.2.6.7	2.2.6.8	2.2.6.9	2.2.7.1	2.2.7.2	2.2.7.3	2.2.7.4	2.2.7.5	2.2.7.6
	2.2.7.7	2.2.7.8	2.2.7.9	2.2.8.1	2.2.8.2	2.2.8.3	2.2.8.4	2.2.8.5	2.2.8.6	2.2.8.7
	2.2.8.8	2.2.8.9	2.2.9.1	2.2.9.2	2.2.9.3	2.2.9.4	2.2.9.5	2.2.9.6	2.2.9.7	2.2.9.8
40	2.2.9.9	2.3.1.1	2.3.1.2	2.3.1.3	2.3.1.4	2.3.1.5	2.3.1.6	2.3.1.7	2.3.1.8	2.3.1.9
	2.3.2.1	2.3.2.2	2.3.2.3	2.3.2.4	2.3.2.5	2.3.2.6	2.3.2.7	2.3.2.8	2.3.2.9	2.3.3.1
	2.3.3.2	2.3.3.3	2.3.3.4	2.3.3.5	2.3.3.6	2.3.3.7	2.3.3.8	2.3.3.9	2.3.4.1	2.3.4.2
	2.3.4.3	2.3.4.4	2.3.4.5	2.3.4.6	2.3.4.7	2.3.4.8	2.3.4.9	2.3.5.1	2.3.5.2	2.3.5.3
	2.3.5.4	2.3.5.5	2.3.5.6	2.3.5.7	2.3.5.8	2.3.5.9	2.3.6.1	2.3.6.2	2.3.6.3	2.3.6.4
45	2.3.6.5	2.3.6.6	2.3.6.7	2.3.6.8	2.3.6.9	2.3.7.1	2.3.7.2	2.3.7.3	2.3.7.4	2.3.7.5
	2.3.7.6	2.3.7.7	2.3.7.8	2.3.7.9	2.3.8.1	2.3.8.2	2.3.8.3	2.3.8.4	2.3.8.5	2.3.8.6
	2.3.8.7	2.3.8.8	2.3.8.9	2.3.9.1	2.3.9.2	2.3.9.3	2.3.9.4	2.3.9.5	2.3.9.6	2.3.9.7
	2.3.9.8	2.3.9.9	2.4.1.1	2.4.1.2	2.4.1.3	2.4.1.4	2.4.1.5	2.4.1.6	2.4.1.7	2.4.1.8
	2.4.1.9	2.4.2.1	2.4.2.2	2.4.2.3	2.4.2.4	2.4.2.5	2.4.2.6	2.4.2.7	2.4.2.8	2.4.2.9
50	2.4.3.1	2.4.3.2	2.4.3.3	2.4.3.4	2.4.3.5	2.4.3.6	2.4.3.7	2.4.3.8	2.4.3.9	2.4.4.1
	2.4.4.2	2.4.4.3	2.4.4.4	2.4.4.5	2.4.4.6	2.4.4.7	2.4.4.8	2.4.4.9	2.4.5.1	2.4.5.2
	2.4.5.3	2.4.5.4	2.4.5.5	2.4.5.6	2.4.5.7	2.4.5.8	2.4.5.9	2.4.6.1	2.4.6.2	2.4.6.3
	2.4.6.4	2.4.6.5	2.4.6.6	2.4.6.7	2.4.6.8	2.4.6.9	2.4.7.1	2.4.7.2	2.4.7.3	2.4.7.4

[illegible]

Table 1 - continued

	3.2.2.3	3.2.2.4	3.2.2.5	3.2.2.6	3.2.2.7	3.2.2.8	3.2.2.9	3.2.3.1	3.2.3.2	3.2.3.3
	3.2.3.4	3.2.3.5	3.2.3.6	3.2.3.7	3.2.3.8	3.2.3.9	3.2.4.1	3.2.4.2	3.2.4.3	3.2.4.4
	3.2.4.5	3.2.4.6	3.2.4.7	3.2.4.8	3.2.4.9	3.2.5.1	3.2.5.2	3.2.5.3	3.2.5.4	3.2.5.5
5	3.2.5.6	3.2.5.7	3.2.5.8	3.2.5.9	3.2.6.1	3.2.6.2	3.2.6.3	3.2.6.4	3.2.6.5	3.2.6.6
	3.2.6.7	3.2.6.8	3.2.6.9	3.2.7.1	3.2.7.2	3.2.7.3	3.2.7.4	3.2.7.5	3.2.7.6	3.2.7.7
	3.2.7.8	3.2.7.9	3.2.8.1	3.2.8.2	3.2.8.3	3.2.8.4	3.2.8.5	3.2.8.6	3.2.8.7	3.2.8.8
	3.2.8.9	3.2.9.1	3.2.9.2	3.2.9.3	3.2.9.4	3.2.9.5	3.2.9.6	3.2.9.7	3.2.9.8	3.2.9.9
	3.3.1.1	3.3.1.2	3.3.1.3	3.3.1.4	3.3.1.5	3.3.1.6	3.3.1.7	3.3.1.8	3.3.1.9	3.3.2.1
10	3.3.2.2	3.3.2.3	3.3.2.4	3.3.2.5	3.3.2.6	3.3.2.7	3.3.2.8	3.3.2.9	3.3.3.1	3.3.3.2
	3.3.3.3	3.3.3.4	3.3.3.5	3.3.3.6	3.3.3.7	3.3.3.8	3.3.3.9	3.3.4.1	3.3.4.2	3.3.4.3
	3.3.4.4	3.3.4.5	3.3.4.6	3.3.4.7	3.3.4.8	3.3.4.9	3.3.5.1	3.3.5.2	3.3.5.3	3.3.5.4
	3.3.5.5	3.3.5.6	3.3.5.7	3.3.5.8	3.3.5.9	3.3.6.1	3.3.6.2	3.3.6.3	3.3.6.4	3.3.6.5
	3.3.6.6	3.3.6.7	3.3.6.8	3.3.6.9	3.3.7.1	3.3.7.2	3.3.7.3	3.3.7.4	3.3.7.5	3.3.7.6
15	3.3.7.7	3.3.7.8	3.3.7.9	3.3.8.1	3.3.8.2	3.3.8.3	3.3.8.4	3.3.8.5	3.3.8.6	3.3.8.7
	3.3.8.8	3.3.8.9	3.3.9.1	3.3.9.2	3.3.9.3	3.3.9.4	3.3.9.5	3.3.9.6	3.3.9.7	3.3.9.8
	3.3.9.9	3.4.1.1	3.4.1.2	3.4.1.3	3.4.1.4	3.4.1.5	3.4.1.6	3.4.1.7	3.4.1.8	3.4.1.9
	3.4.2.1	3.4.2.2	3.4.2.3	3.4.2.4	3.4.2.5	3.4.2.6	3.4.2.7	3.4.2.8	3.4.2.9	3.4.3.1
	3.4.3.2	3.4.3.3	3.4.3.4	3.4.3.5	3.4.3.6	3.4.3.7	3.4.3.8	3.4.3.9	3.4.4.1	3.4.4.2
20	3.4.4.3	3.4.4.4	3.4.4.5	3.4.4.6	3.4.4.7	3.4.4.8	3.4.4.9	3.4.5.1	3.4.5.2	3.4.5.3
	3.4.5.4	3.4.5.5	3.4.5.6	3.4.5.7	3.4.5.8	3.4.5.9	3.4.6.1	3.4.6.2	3.4.6.3	3.4.6.4
	3.4.6.5	3.4.6.6	3.4.6.7	3.4.6.8	3.4.6.9	3.4.7.1	3.4.7.2	3.4.7.3	3.4.7.4	3.4.7.5
	3.4.7.6	3.4.7.7	3.4.7.8	3.4.7.9	3.4.8.1	3.4.8.2	3.4.8.3	3.4.8.4	3.4.8.5	3.4.8.6
	3.4.8.7	3.4.8.8	3.4.8.9	3.4.9.1	3.4.9.2	3.4.9.3	3.4.9.4	3.4.9.5	3.4.9.6	3.4.9.7
25	3.4.9.8	3.4.9.9	3.5.1.1	3.5.1.2	3.5.1.3	3.5.1.4	3.5.1.5	3.5.1.6	3.5.1.7	3.5.1.8
	3.5.1.9	3.5.2.1	3.5.2.2	3.5.2.3	3.5.2.4	3.5.2.5	3.5.2.6	3.5.2.7	3.5.2.8	3.5.2.9
	3.5.3.1	3.5.3.2	3.5.3.3	3.5.3.4	3.5.3.5	3.5.3.6	3.5.3.7	3.5.3.8	3.5.3.9	3.5.4.1
	3.5.4.2	3.5.4.3	3.5.4.4	3.5.4.5	3.5.4.6	3.5.4.7	3.5.4.8	3.5.4.9	3.5.5.1	3.5.5.2
	3.5.5.3	3.5.5.4	3.5.5.5	3.5.5.6	3.5.5.7	3.5.5.8	3.5.5.9	3.5.6.1	3.5.6.2	3.5.6.3
30	3.5.6.4	3.5.6.5	3.5.6.6	3.5.6.7	3.5.6.8	3.5.6.9	3.5.7.1	3.5.7.2	3.5.7.3	3.5.7.4
	3.5.7.5	3.5.7.6	3.5.7.7	3.5.7.8	3.5.7.9	3.5.8.1	3.5.8.2	3.5.8.3	3.5.8.4	3.5.8.5
	3.5.8.6	3.5.8.7	3.5.8.8	3.5.8.9	3.5.9.1	3.5.9.2	3.5.9.3	3.5.9.4	3.5.9.5	3.5.9.6
	3.5.9.7	3.5.9.8	3.5.9.9	3.6.1.1	3.6.1.2	3.6.1.3	3.6.1.4	3.6.1.5	3.6.1.6	3.6.1.7
	3.6.1.8	3.6.1.9	3.6.2.1	3.6.2.2	3.6.2.3	3.6.2.4	3.6.2.5	3.6.2.6	3.6.2.7	3.6.2.8
35	3.6.2.9	3.6.3.1	3.6.3.2	3.6.3.3	3.6.3.4	3.6.3.5	3.6.3.6	3.6.3.7	3.6.3.8	3.6.3.9
	3.6.4.1	3.6.4.2	3.6.4.3	3.6.4.4	3.6.4.5	3.6.4.6	3.6.4.7	3.6.4.8	3.6.4.9	3.6.5.1
	3.6.5.2	3.6.5.3	3.6.5.4	3.6.5.5	3.6.5.6	3.6.5.7	3.6.5.8	3.6.5.9	3.6.6.1	3.6.6.2
	3.6.6.3	3.6.6.4	3.6.6.5	3.6.6.6	3.6.6.7	3.6.6.8	3.6.6.9	3.6.7.1	3.6.7.2	3.6.7.3
	3.6.7.4	3.6.7.5	3.6.7.6	3.6.7.7	3.6.7.8	3.6.7.9	3.6.8.1	3.6.8.2	3.6.8.3	3.6.8.4
40	3.6.8.5	3.6.8.6	3.6.8.7	3.6.8.8	3.6.8.9	3.6.9.1	3.6.9.2	3.6.9.3	3.6.9.4	3.6.9.5
	3.6.9.6	3.6.9.7	3.6.9.8	3.6.9.9	3.7.1.1	3.7.1.2	3.7.1.3	3.7.1.4	3.7.1.5	3.7.1.6
	3.7.1.7	3.7.1.8	3.7.1.9	3.7.2.1	3.7.2.2	3.7.2.3	3.7.2.4	3.7.2.5	3.7.2.6	3.7.2.7
	3.7.2.8	3.7.2.9	3.7.3.1	3.7.3.2	3.7.3.3	3.7.3.4	3.7.3.5	3.7.3.6	3.7.3.7	3.7.3.8
	3.7.3.9	3.7.4.1	3.7.4.2	3.7.4.3	3.7.4.4	3.7.4.5	3.7.4.6	3.7.4.7	3.7.4.8	3.7.4.9
45	3.7.5.1	3.7.5.2	3.7.5.3	3.7.5.4	3.7.5.5	3.7.5.6	3.7.5.7	3.7.5.8	3.7.5.9	3.7.6.1
	3.7.6.2	3.7.6.3	3.7.6.4	3.7.6.5	3.7.6.6	3.7.6.7	3.7.6.8	3.7.6.9	3.7.7.1	3.7.7.2
	3.7.7.3	3.7.7.4	3.7.7.5	3.7.7.6	3.7.7.7	3.7.7.8	3.7.7.9	3.7.8.1	3.7.8.2	3.7.8.3
	3.7.8.4	3.7.8.5	3.7.8.6	3.7.8.7	3.7.8.8	3.7.8.9	3.7.9.1	3.7.9.2	3.7.9.3	3.7.9.4
	3.7.9.5	3.7.9.6	3.7.9.7	3.7.9.8	3.7.9.9	3.8.1.1	3.8.1.2	3.8.1.3	3.8.1.4	3.8.1.5
50	3.8.1.6	3.8.1.7	3.8.1.8	3.8.1.9	3.8.2.1	3.8.2.2	3.8.2.3	3.8.2.4	3.8.2.5	3.8.2.6
	3.8.2.7	3.8.2.8	3.8.2.9	3.8.3.1	3.8.3.2	3.8.3.3	3.8.3.4	3.8.3.5	3.8.3.6	3.8.3.7
	3.8.3.8	3.8.3.9	3.8.4.1	3.8.4.2	3.8.4.3	3.8.4.4	3.8.4.5	3.8.4.6	3.8.4.7	3.8.4.8
	3.8.4.9	3.8.5.1	3.8.5.2	3.8.5.3	3.8.5.4	3.8.5.5	3.8.5.6	3.8.5.7	3.8.5.8	3.8.5.9

09518501-030300

Table 1 - continued

	3.8.6.1	3.8.6.2	3.8.6.3	3.8.6.4	3.8.6.5	3.8.6.6	3.8.6.7	3.8.6.8	3.8.6.9	3.8.7.1
	3.8.7.2	3.8.7.3	3.8.7.4	3.8.7.5	3.8.7.6	3.8.7.7	3.8.7.8	3.8.7.9	3.8.8.1	3.8.8.2
	3.8.8.3	3.8.8.4	3.8.8.5	3.8.8.6	3.8.8.7	3.8.8.8	3.8.8.9	3.8.9.1	3.8.9.2	3.8.9.3
5	3.8.9.4	3.8.9.5	3.8.9.6	3.8.9.7	3.8.9.8	3.8.9.9	3.9.1.1	3.9.1.2	3.9.1.3	3.9.1.4
	3.9.1.5	3.9.1.6	3.9.1.7	3.9.1.8	3.9.1.9	3.9.2.1	3.9.2.2	3.9.2.3	3.9.2.4	3.9.2.5
	3.9.2.6	3.9.2.7	3.9.2.8	3.9.2.9	3.9.3.1	3.9.3.2	3.9.3.3	3.9.3.4	3.9.3.5	3.9.3.6
	3.9.3.7	3.9.3.8	3.9.3.9	3.9.4.1	3.9.4.2	3.9.4.3	3.9.4.4	3.9.4.5	3.9.4.6	3.9.4.7
	3.9.4.8	3.9.4.9	3.9.5.1	3.9.5.2	3.9.5.3	3.9.5.4	3.9.5.5	3.9.5.6	3.9.5.7	3.9.5.8
10	3.9.5.9	3.9.6.1	3.9.6.2	3.9.6.3	3.9.6.4	3.9.6.5	3.9.6.6	3.9.6.7	3.9.6.8	3.9.6.9
	3.9.7.1	3.9.7.2	3.9.7.3	3.9.7.4	3.9.7.5	3.9.7.6	3.9.7.7	3.9.7.8	3.9.7.9	3.9.8.1
	3.9.8.2	3.9.8.3	3.9.8.4	3.9.8.5	3.9.8.6	3.9.8.7	3.9.8.8	3.9.8.9	3.9.9.1	3.9.9.2
	3.9.9.3	3.9.9.4	3.9.9.5	3.9.9.6	3.9.9.7	3.9.9.8	3.9.9.9	4.1.1.1	4.1.1.2	4.1.1.3
	4.1.1.4	4.1.1.5	4.1.1.6	4.1.1.7	4.1.1.8	4.1.1.9	4.1.2.1	4.1.2.2	4.1.2.3	4.1.2.4
15	4.1.2.5	4.1.2.6	4.1.2.7	4.1.2.8	4.1.2.9	4.1.3.1	4.1.3.2	4.1.3.3	4.1.3.4	4.1.3.5
	4.1.3.6	4.1.3.7	4.1.3.8	4.1.3.9	4.1.4.1	4.1.4.2	4.1.4.3	4.1.4.4	4.1.4.5	4.1.4.6
	4.1.4.7	4.1.4.8	4.1.4.9	4.1.5.1	4.1.5.2	4.1.5.3	4.1.5.4	4.1.5.5	4.1.5.6	4.1.5.7
	4.1.5.8	4.1.5.9	4.1.6.1	4.1.6.2	4.1.6.3	4.1.6.4	4.1.6.5	4.1.6.6	4.1.6.7	4.1.6.8
	4.1.6.9	4.1.7.1	4.1.7.2	4.1.7.3	4.1.7.4	4.1.7.5	4.1.7.6	4.1.7.7	4.1.7.8	4.1.7.9
20	4.1.8.1	4.1.8.2	4.1.8.3	4.1.8.4	4.1.8.5	4.1.8.6	4.1.8.7	4.1.8.8	4.1.8.9	4.1.9.1
	4.1.9.2	4.1.9.3	4.1.9.4	4.1.9.5	4.1.9.6	4.1.9.7	4.1.9.8	4.1.9.9	4.2.1.1	4.2.1.2
	4.2.1.3	4.2.1.4	4.2.1.5	4.2.1.6	4.2.1.7	4.2.1.8	4.2.1.9	4.2.2.1	4.2.2.2	4.2.2.3
	4.2.2.4	4.2.2.5	4.2.2.6	4.2.2.7	4.2.2.8	4.2.2.9	4.2.3.1	4.2.3.2	4.2.3.3	4.2.3.4
	4.2.3.5	4.2.3.6	4.2.3.7	4.2.3.8	4.2.3.9	4.2.4.1	4.2.4.2	4.2.4.3	4.2.4.4	4.2.4.5
25	4.2.4.6	4.2.4.7	4.2.4.8	4.2.4.9	4.2.5.1	4.2.5.2	4.2.5.3	4.2.5.4	4.2.5.5	4.2.5.6
	4.2.5.7	4.2.5.8	4.2.5.9	4.2.6.1	4.2.6.2	4.2.6.3	4.2.6.4	4.2.6.5	4.2.6.6	4.2.6.7
	4.2.6.8	4.2.6.9	4.2.7.1	4.2.7.2	4.2.7.3	4.2.7.4	4.2.7.5	4.2.7.6	4.2.7.7	4.2.7.8
	4.2.7.9	4.2.8.1	4.2.8.2	4.2.8.3	4.2.8.4	4.2.8.5	4.2.8.6	4.2.8.7	4.2.8.8	4.2.8.9
	4.2.9.1	4.2.9.2	4.2.9.3	4.2.9.4	4.2.9.5	4.2.9.6	4.2.9.7	4.2.9.8	4.2.9.9	4.3.1.1
30	4.3.1.2	4.3.1.3	4.3.1.4	4.3.1.5	4.3.1.6	4.3.1.7	4.3.1.8	4.3.1.9	4.3.2.1	4.3.2.2
	4.3.2.3	4.3.2.4	4.3.2.5	4.3.2.6	4.3.2.7	4.3.2.8	4.3.2.9	4.3.3.1	4.3.3.2	4.3.3.3
	4.3.3.4	4.3.3.5	4.3.3.6	4.3.3.7	4.3.3.8	4.3.3.9	4.3.4.1	4.3.4.2	4.3.4.3	4.3.4.4
	4.3.4.5	4.3.4.6	4.3.4.7	4.3.4.8	4.3.4.9	4.3.5.1	4.3.5.2	4.3.5.3	4.3.5.4	4.3.5.5
	4.3.5.6	4.3.5.7	4.3.5.8	4.3.5.9	4.3.6.1	4.3.6.2	4.3.6.3	4.3.6.4	4.3.6.5	4.3.6.6
35	4.3.6.7	4.3.6.8	4.3.6.9	4.3.7.1	4.3.7.2	4.3.7.3	4.3.7.4	4.3.7.5	4.3.7.6	4.3.7.7
	4.3.7.8	4.3.7.9	4.3.8.1	4.3.8.2	4.3.8.3	4.3.8.4	4.3.8.5	4.3.8.6	4.3.8.7	4.3.8.8
	4.3.8.9	4.3.9.1	4.3.9.2	4.3.9.3	4.3.9.4	4.3.9.5	4.3.9.6	4.3.9.7	4.3.9.8	4.3.9.9
	4.4.1.1	4.4.1.2	4.4.1.3	4.4.1.4	4.4.1.5	4.4.1.6	4.4.1.7	4.4.1.8	4.4.1.9	4.4.2.1
	4.4.2.2	4.4.2.3	4.4.2.4	4.4.2.5	4.4.2.6	4.4.2.7	4.4.2.8	4.4.2.9	4.4.3.1	4.4.3.2
40	4.4.3.3	4.4.3.4	4.4.3.5	4.4.3.6	4.4.3.7	4.4.3.8	4.4.3.9	4.4.4.1	4.4.4.2	4.4.4.3
	4.4.4.4	4.4.4.5	4.4.4.6	4.4.4.7	4.4.4.8	4.4.4.9	4.4.5.1	4.4.5.2	4.4.5.3	4.4.5.4
	4.4.5.5	4.4.5.6	4.4.5.7	4.4.5.8	4.4.5.9	4.4.6.1	4.4.6.2	4.4.6.3	4.4.6.4	4.4.6.5
	4.4.6.6	4.4.6.7	4.4.6.8	4.4.6.9	4.4.7.1	4.4.7.2	4.4.7.3	4.4.7.4	4.4.7.5	4.4.7.6
	4.4.7.7	4.4.7.8	4.4.7.9	4.4.8.1	4.4.8.2	4.4.8.3	4.4.8.4	4.4.8.5	4.4.8.6	4.4.8.7
45	4.4.8.8	4.4.8.9	4.4.9.1	4.4.9.2	4.4.9.3	4.4.9.4	4.4.9.5	4.4.9.6	4.4.9.7	4.4.9.8
	4.4.9.9	4.5.1.1	4.5.1.2	4.5.1.3	4.5.1.4	4.5.1.5	4.5.1.6	4.5.1.7	4.5.1.8	4.5.1.9
	4.5.2.1	4.5.2.2	4.5.2.3	4.5.2.4	4.5.2.5	4.5.2.6	4.5.2.7	4.5.2.8	4.5.2.9	4.5.3.1
	4.5.3.2	4.5.3.3	4.5.3.4	4.5.3.5	4.5.3.6	4.5.3.7	4.5.3.8	4.5.3.9	4.5.4.1	4.5.4.2
	4.5.4.3	4.5.4.4	4.5.4.5	4.5.4.6	4.5.4.7	4.5.4.8	4.5.4.9	4.5.5.1	4.5.5.2	4.5.5.3
50	4.5.5.4	4.5.5.5	4.5.5.6	4.5.5.7	4.5.5.8	4.5.5.9	4.5.6.1	4.5.6.2	4.5.6.3	4.5.6.4
	4.5.6.5	4.5.6.6	4.5.6.7	4.5.6.8	4.5.6.9	4.5.7.1	4.5.7.2	4.5.7.3	4.5.7.4	4.5.7.5
	4.5.7.6	4.5.7.7	4.5.7.8	4.5.7.9	4.5.8.1	4.5.8.2	4.5.8.3	4.5.8.4	4.5.8.5	4.5.8.6
	4.5.8.7	4.5.8.8	4.5.8.9	4.5.9.1	4.5.9.2	4.5.9.3	4.5.9.4	4.5.9.5	4.5.9.6	4.5.9.7

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Table 1 - continued

	4.5.9.8	4.5.9.9	4.6.1.1	4.6.1.2	4.6.1.3	4.6.1.4	4.6.1.5	4.6.1.6	4.6.1.7	4.6.1.8
	4.6.1.9	4.6.2.1	4.6.2.2	4.6.2.3	4.6.2.4	4.6.2.5	4.6.2.6	4.6.2.7	4.6.2.8	4.6.2.9
	4.6.3.1	4.6.3.2	4.6.3.3	4.6.3.4	4.6.3.5	4.6.3.6	4.6.3.7	4.6.3.8	4.6.3.9	4.6.4.1
5	4.6.4.2	4.6.4.3	4.6.4.4	4.6.4.5	4.6.4.6	4.6.4.7	4.6.4.8	4.6.4.9	4.6.5.1	4.6.5.2
	4.6.5.3	4.6.5.4	4.6.5.5	4.6.5.6	4.6.5.7	4.6.5.8	4.6.5.9	4.6.6.1	4.6.6.2	4.6.6.3
	4.6.6.4	4.6.6.5	4.6.6.6	4.6.6.7	4.6.6.8	4.6.6.9	4.6.7.1	4.6.7.2	4.6.7.3	4.6.7.4
	4.6.7.5	4.6.7.6	4.6.7.7	4.6.7.8	4.6.7.9	4.6.8.1	4.6.8.2	4.6.8.3	4.6.8.4	4.6.8.5
	4.6.8.6	4.6.8.7	4.6.8.8	4.6.8.9	4.6.9.1	4.6.9.2	4.6.9.3	4.6.9.4	4.6.9.5	4.6.9.6
10	4.6.9.7	4.6.9.8	4.6.9.9	4.7.1.1	4.7.1.2	4.7.1.3	4.7.1.4	4.7.1.5	4.7.1.6	4.7.1.7
	4.7.1.8	4.7.1.9	4.7.2.1	4.7.2.2	4.7.2.3	4.7.2.4	4.7.2.5	4.7.2.6	4.7.2.7	4.7.2.8
	4.7.2.9	4.7.3.1	4.7.3.2	4.7.3.3	4.7.3.4	4.7.3.5	4.7.3.6	4.7.3.7	4.7.3.8	4.7.3.9
	4.7.4.1	4.7.4.2	4.7.4.3	4.7.4.4	4.7.4.5	4.7.4.6	4.7.4.7	4.7.4.8	4.7.4.9	4.7.5.1
	4.7.5.2	4.7.5.3	4.7.5.4	4.7.5.5	4.7.5.6	4.7.5.7	4.7.5.8	4.7.5.9	4.7.6.1	4.7.6.2
15	4.7.6.3	4.7.6.4	4.7.6.5	4.7.6.6	4.7.6.7	4.7.6.8	4.7.6.9	4.7.7.1	4.7.7.2	4.7.7.3
	4.7.7.4	4.7.7.5	4.7.7.6	4.7.7.7	4.7.7.8	4.7.7.9	4.7.8.1	4.7.8.2	4.7.8.3	4.7.8.4
	4.7.8.5	4.7.8.6	4.7.8.7	4.7.8.8	4.7.8.9	4.7.9.1	4.7.9.2	4.7.9.3	4.7.9.4	4.7.9.5
	4.7.9.6	4.7.9.7	4.7.9.8	4.7.9.9	4.8.1.1	4.8.1.2	4.8.1.3	4.8.1.4	4.8.1.5	4.8.1.6
	4.8.1.7	4.8.1.8	4.8.1.9	4.8.2.1	4.8.2.2	4.8.2.3	4.8.2.4	4.8.2.5	4.8.2.6	4.8.2.7
20	4.8.2.8	4.8.2.9	4.8.3.1	4.8.3.2	4.8.3.3	4.8.3.4	4.8.3.5	4.8.3.6	4.8.3.7	4.8.3.8
	4.8.3.9	4.8.4.1	4.8.4.2	4.8.4.3	4.8.4.4	4.8.4.5	4.8.4.6	4.8.4.7	4.8.4.8	4.8.4.9
	4.8.5.1	4.8.5.2	4.8.5.3	4.8.5.4	4.8.5.5	4.8.5.6	4.8.5.7	4.8.5.8	4.8.5.9	4.8.6.1
	4.8.6.2	4.8.6.3	4.8.6.4	4.8.6.5	4.8.6.6	4.8.6.7	4.8.6.8	4.8.6.9	4.8.7.1	4.8.7.2
	4.8.7.3	4.8.7.4	4.8.7.5	4.8.7.6	4.8.7.7	4.8.7.8	4.8.7.9	4.8.8.1	4.8.8.2	4.8.8.3
25	4.8.8.4	4.8.8.5	4.8.8.6	4.8.8.7	4.8.8.8	4.8.8.9	4.8.9.1	4.8.9.2	4.8.9.3	4.8.9.4
	4.8.9.5	4.8.9.6	4.8.9.7	4.8.9.8	4.8.9.9	4.9.1.1	4.9.1.2	4.9.1.3	4.9.1.4	4.9.1.5
	4.9.1.6	4.9.1.7	4.9.1.8	4.9.1.9	4.9.2.1	4.9.2.2	4.9.2.3	4.9.2.4	4.9.2.5	4.9.2.6
	4.9.2.7	4.9.2.8	4.9.2.9	4.9.3.1	4.9.3.2	4.9.3.3	4.9.3.4	4.9.3.5	4.9.3.6	4.9.3.7
	4.9.3.8	4.9.3.9	4.9.4.1	4.9.4.2	4.9.4.3	4.9.4.4	4.9.4.5	4.9.4.6	4.9.4.7	4.9.4.8
30	4.9.4.9	4.9.5.1	4.9.5.2	4.9.5.3	4.9.5.4	4.9.5.5	4.9.5.6	4.9.5.7	4.9.5.8	4.9.5.9
	4.9.6.1	4.9.6.2	4.9.6.3	4.9.6.4	4.9.6.5	4.9.6.6	4.9.6.7	4.9.6.8	4.9.6.9	4.9.7.1
	4.9.7.2	4.9.7.3	4.9.7.4	4.9.7.5	4.9.7.6	4.9.7.7	4.9.7.8	4.9.7.9	4.9.8.1	4.9.8.2
	4.9.8.3	4.9.8.4	4.9.8.5	4.9.8.6	4.9.8.7	4.9.8.8	4.9.8.9	4.9.9.1	4.9.9.2	4.9.9.3
	4.9.9.4	4.9.9.5	4.9.9.6	4.9.9.7	4.9.9.8	4.9.9.9	5.1.1.1	5.1.1.2	5.1.1.3	5.1.1.4
35	5.1.1.5	5.1.1.6	5.1.1.7	5.1.1.8	5.1.1.9	5.1.2.1	5.1.2.2	5.1.2.3	5.1.2.4	5.1.2.5
	5.1.2.6	5.1.2.7	5.1.2.8	5.1.2.9	5.1.3.1	5.1.3.2	5.1.3.3	5.1.3.4	5.1.3.5	5.1.3.6
	5.1.3.7	5.1.3.8	5.1.3.9	5.1.4.1	5.1.4.2	5.1.4.3	5.1.4.4	5.1.4.5	5.1.4.6	5.1.4.7
	5.1.4.8	5.1.4.9	5.1.5.1	5.1.5.2	5.1.5.3	5.1.5.4	5.1.5.5	5.1.5.6	5.1.5.7	5.1.5.8
	5.1.5.9	5.1.6.1	5.1.6.2	5.1.6.3	5.1.6.4	5.1.6.5	5.1.6.6	5.1.6.7	5.1.6.8	5.1.6.9
40	5.1.7.1	5.1.7.2	5.1.7.3	5.1.7.4	5.1.7.5	5.1.7.6	5.1.7.7	5.1.7.8	5.1.7.9	5.1.8.1
	5.1.8.2	5.1.8.3	5.1.8.4	5.1.8.5	5.1.8.6	5.1.8.7	5.1.8.8	5.1.8.9	5.1.9.1	5.1.9.2
	5.1.9.3	5.1.9.4	5.1.9.5	5.1.9.6	5.1.9.7	5.1.9.8	5.1.9.9	5.2.1.1	5.2.1.2	5.2.1.3
	5.2.1.4	5.2.1.5	5.2.1.6	5.2.1.7	5.2.1.8	5.2.1.9	5.2.2.1	5.2.2.2	5.2.2.3	5.2.2.4
	5.2.2.5	5.2.2.6	5.2.2.7	5.2.2.8	5.2.2.9	5.2.3.1	5.2.3.2	5.2.3.3	5.2.3.4	5.2.3.5
45	5.2.3.6	5.2.3.7	5.2.3.8	5.2.3.9	5.2.4.1	5.2.4.2	5.2.4.3	5.2.4.4	5.2.4.5	5.2.4.6
	5.2.4.7	5.2.4.8	5.2.4.9	5.2.5.1	5.2.5.2	5.2.5.3	5.2.5.4	5.2.5.5	5.2.5.6	5.2.5.7
	5.2.5.8	5.2.5.9	5.2.6.1	5.2.6.2	5.2.6.3	5.2.6.4	5.2.6.5	5.2.6.6	5.2.6.7	5.2.6.8
	5.2.6.9	5.2.7.1	5.2.7.2	5.2.7.3	5.2.7.4	5.2.7.5	5.2.7.6	5.2.7.7	5.2.7.8	5.2.7.9
	5.2.8.1	5.2.8.2	5.2.8.3	5.2.8.4	5.2.8.5	5.2.8.6	5.2.8.7	5.2.8.8	5.2.8.9	5.2.9.1
50	5.2.9.2	5.2.9.3	5.2.9.4	5.2.9.5	5.2.9.6	5.2.9.7	5.2.9.8	5.2.9.9	5.3.1.1	5.3.1.2
	5.3.1.3	5.3.1.4	5.3.1.5	5.3.1.6	5.3.1.7	5.3.1.8	5.3.1.9	5.3.2.1	5.3.2.2	5.3.2.3
	5.3.2.4	5.3.2.5	5.3.2.6	5.3.2.7	5.3.2.8	5.3.2.9	5.3.3.1	5.3.3.2	5.3.3.3	5.3.3.4
	5.3.3.5	5.3.3.6	5.3.3.7	5.3.3.8	5.3.3.9	5.3.4.1	5.3.4.2	5.3.4.3	5.3.4.4	5.3.4.5

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[illegible]

Table 1 - continued

	5.9.8.4	5.9.8.5	5.9.8.6	5.9.8.7	5.9.8.8	5.9.8.9	5.9.9.1	5.9.9.2	5.9.9.3	5.9.9.4
	5.9.9.5	5.9.9.6	5.9.9.7	5.9.9.8	5.9.9.9	6.1.1.1	6.1.1.2	6.1.1.3	6.1.1.4	6.1.1.5
	6.1.1.6	6.1.1.7	6.1.1.8	6.1.1.9	6.1.2.1	6.1.2.2	6.1.2.3	6.1.2.4	6.1.2.5	6.1.2.6
5	6.1.2.7	6.1.2.8	6.1.2.9	6.1.3.1	6.1.3.2	6.1.3.3	6.1.3.4	6.1.3.5	6.1.3.6	6.1.3.7
	6.1.3.8	6.1.3.9	6.1.4.1	6.1.4.2	6.1.4.3	6.1.4.4	6.1.4.5	6.1.4.6	6.1.4.7	6.1.4.8
	6.1.4.9	6.1.5.1	6.1.5.2	6.1.5.3	6.1.5.4	6.1.5.5	6.1.5.6	6.1.5.7	6.1.5.8	6.1.5.9
	6.1.6.1	6.1.6.2	6.1.6.3	6.1.6.4	6.1.6.5	6.1.6.6	6.1.6.7	6.1.6.8	6.1.6.9	6.1.7.1
	6.1.7.2	6.1.7.3	6.1.7.4	6.1.7.5	6.1.7.6	6.1.7.7	6.1.7.8	6.1.7.9	6.1.8.1	6.1.8.2
10	6.1.8.3	6.1.8.4	6.1.8.5	6.1.8.6	6.1.8.7	6.1.8.8	6.1.8.9	6.1.9.1	6.1.9.2	6.1.9.3
	6.1.9.4	6.1.9.5	6.1.9.6	6.1.9.7	6.1.9.8	6.1.9.9	6.2.1.1	6.2.1.2	6.2.1.3	6.2.1.4
	6.2.1.5	6.2.1.6	6.2.1.7	6.2.1.8	6.2.1.9	6.2.2.1	6.2.2.2	6.2.2.3	6.2.2.4	6.2.2.5
	6.2.2.6	6.2.2.7	6.2.2.8	6.2.2.9	6.2.3.1	6.2.3.2	6.2.3.3	6.2.3.4	6.2.3.5	6.2.3.6
	6.2.3.7	6.2.3.8	6.2.3.9	6.2.4.1	6.2.4.2	6.2.4.3	6.2.4.4	6.2.4.5	6.2.4.6	6.2.4.7
15	6.2.4.8	6.2.4.9	6.2.5.1	6.2.5.2	6.2.5.3	6.2.5.4	6.2.5.5	6.2.5.6	6.2.5.7	6.2.5.8
	6.2.5.9	6.2.6.1	6.2.6.2	6.2.6.3	6.2.6.4	6.2.6.5	6.2.6.6	6.2.6.7	6.2.6.8	6.2.6.9
	6.2.7.1	6.2.7.2	6.2.7.3	6.2.7.4	6.2.7.5	6.2.7.6	6.2.7.7	6.2.7.8	6.2.7.9	6.2.8.1
	6.2.8.2	6.2.8.3	6.2.8.4	6.2.8.5	6.2.8.6	6.2.8.7	6.2.8.8	6.2.8.9	6.2.9.1	6.2.9.2
	6.2.9.3	6.2.9.4	6.2.9.5	6.2.9.6	6.2.9.7	6.2.9.8	6.2.9.9	6.3.1.1	6.3.1.2	6.3.1.3
20	6.3.1.4	6.3.1.5	6.3.1.6	6.3.1.7	6.3.1.8	6.3.1.9	6.3.2.1	6.3.2.2	6.3.2.3	6.3.2.4
	6.3.2.5	6.3.2.6	6.3.2.7	6.3.2.8	6.3.2.9	6.3.3.1	6.3.3.2	6.3.3.3	6.3.3.4	6.3.3.5
	6.3.3.6	6.3.3.7	6.3.3.8	6.3.3.9	6.3.4.1	6.3.4.2	6.3.4.3	6.3.4.4	6.3.4.5	6.3.4.6
	6.3.4.7	6.3.4.8	6.3.4.9	6.3.5.1	6.3.5.2	6.3.5.3	6.3.5.4	6.3.5.5	6.3.5.6	6.3.5.7
	6.3.5.8	6.3.5.9	6.3.6.1	6.3.6.2	6.3.6.3	6.3.6.4	6.3.6.5	6.3.6.6	6.3.6.7	6.3.6.8
25	6.3.6.9	6.3.7.1	6.3.7.2	6.3.7.3	6.3.7.4	6.3.7.5	6.3.7.6	6.3.7.7	6.3.7.8	6.3.7.9
	6.3.8.1	6.3.8.2	6.3.8.3	6.3.8.4	6.3.8.5	6.3.8.6	6.3.8.7	6.3.8.8	6.3.8.9	6.3.9.1
	6.3.9.2	6.3.9.3	6.3.9.4	6.3.9.5	6.3.9.6	6.3.9.7	6.3.9.8	6.3.9.9	6.4.1.1	6.4.1.2
	6.4.1.3	6.4.1.4	6.4.1.5	6.4.1.6	6.4.1.7	6.4.1.8	6.4.1.9	6.4.2.1	6.4.2.2	6.4.2.3
	6.4.2.4	6.4.2.5	6.4.2.6	6.4.2.7	6.4.2.8	6.4.2.9	6.4.3.1	6.4.3.2	6.4.3.3	6.4.3.4
30	6.4.3.5	6.4.3.6	6.4.3.7	6.4.3.8	6.4.3.9	6.4.4.1	6.4.4.2	6.4.4.3	6.4.4.4	6.4.4.5
	6.4.4.6	6.4.4.7	6.4.4.8	6.4.4.9	6.4.5.1	6.4.5.2	6.4.5.3	6.4.5.4	6.4.5.5	6.4.5.6
	6.4.5.7	6.4.5.8	6.4.5.9	6.4.6.1	6.4.6.2	6.4.6.3	6.4.6.4	6.4.6.5	6.4.6.6	6.4.6.7
	6.4.6.8	6.4.6.9	6.4.7.1	6.4.7.2	6.4.7.3	6.4.7.4	6.4.7.5	6.4.7.6	6.4.7.7	6.4.7.8
	6.4.7.9	6.4.8.1	6.4.8.2	6.4.8.3	6.4.8.4	6.4.8.5	6.4.8.6	6.4.8.7	6.4.8.8	6.4.8.9
35	6.4.9.1	6.4.9.2	6.4.9.3	6.4.9.4	6.4.9.5	6.4.9.6	6.4.9.7	6.4.9.8	6.4.9.9	6.5.1.1
	6.5.1.2	6.5.1.3	6.5.1.4	6.5.1.5	6.5.1.6	6.5.1.7	6.5.1.8	6.5.1.9	6.5.2.1	6.5.2.2
	6.5.2.3	6.5.2.4	6.5.2.5	6.5.2.6	6.5.2.7	6.5.2.8	6.5.2.9	6.5.3.1	6.5.3.2	6.5.3.3
	6.5.3.4	6.5.3.5	6.5.3.6	6.5.3.7	6.5.3.8	6.5.3.9	6.5.4.1	6.5.4.2	6.5.4.3	6.5.4.4
	6.5.4.5	6.5.4.6	6.5.4.7	6.5.4.8	6.5.4.9	6.5.5.1	6.5.5.2	6.5.5.3	6.5.5.4	6.5.5.5
40	6.5.5.6	6.5.5.7	6.5.5.8	6.5.5.9	6.5.6.1	6.5.6.2	6.5.6.3	6.5.6.4	6.5.6.5	6.5.6.6
	6.5.6.7	6.5.6.8	6.5.6.9	6.5.7.1	6.5.7.2	6.5.7.3	6.5.7.4	6.5.7.5	6.5.7.6	6.5.7.7
	6.5.7.8	6.5.7.9	6.5.8.1	6.5.8.2	6.5.8.3	6.5.8.4	6.5.8.5	6.5.8.6	6.5.8.7	6.5.8.8
	6.5.8.9	6.5.9.1	6.5.9.2	6.5.9.3	6.5.9.4	6.5.9.5	6.5.9.6	6.5.9.7	6.5.9.8	6.5.9.9
	6.6.1.1	6.6.1.2	6.6.1.3	6.6.1.4	6.6.1.5	6.6.1.6	6.6.1.7	6.6.1.8	6.6.1.9	6.6.2.1
45	6.6.2.2	6.6.2.3	6.6.2.4	6.6.2.5	6.6.2.6	6.6.2.7	6.6.2.8	6.6.2.9	6.6.3.1	6.6.3.2
	6.6.3.3	6.6.3.4	6.6.3.5	6.6.3.6	6.6.3.7	6.6.3.8	6.6.3.9	6.6.4.1	6.6.4.2	6.6.4.3
	6.6.4.4	6.6.4.5	6.6.4.6	6.6.4.7	6.6.4.8	6.6.4.9	6.6.5.1	6.6.5.2	6.6.5.3	6.6.5.4
	6.6.5.5	6.6.5.6	6.6.5.7	6.6.5.8	6.6.5.9	6.6.6.1	6.6.6.2	6.6.6.3	6.6.6.4	6.6.6.5
	6.6.6.6	6.6.6.7	6.6.6.8	6.6.6.9	6.6.7.1	6.6.7.2	6.6.7.3	6.6.7.4	6.6.7.5	6.6.7.6
50	6.6.7.7	6.6.7.8	6.6.7.9	6.6.8.1	6.6.8.2	6.6.8.3	6.6.8.4	6.6.8.5	6.6.8.6	6.6.8.7
	6.6.8.8	6.6.8.9	6.6.9.1	6.6.9.2	6.6.9.3	6.6.9.4	6.6.9.5	6.6.9.6	6.6.9.7	6.6.9.8
	6.6.9.9	6.7.1.1	6.7.1.2	6.7.1.3	6.7.1.4	6.7.1.5	6.7.1.6	6.7.1.7	6.7.1.8	6.7.1.9
	6.7.2.1	6.7.2.2	6.7.2.3	6.7.2.4	6.7.2.5	6.7.2.6	6.7.2.7	6.7.2.8	6.7.2.9	6.7.3.1

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6.7.3.2	6.7.3.3	6.7.3.4	6.7.3.5	6.7.3.6	6.7.3.7	6.7.3.8	6.7.3.9	6.7.4.1	6.7.4.2
6.7.4.3	6.7.4.4	6.7.4.5	6.7.4.6	6.7.4.7	6.7.4.8	6.7.4.9	6.7.5.1	6.7.5.2	6.7.5.3
6.7.5.4	6.7.5.5	6.7.5.6	6.7.5.7	6.7.5.8	6.7.5.9	6.7.6.1	6.7.6.2	6.7.6.3	6.7.6.4
6.7.6.5	6.7.6.6	6.7.6.7	6.7.6.8	6.7.6.9	6.7.7.1	6.7.7.2	6.7.7.3	6.7.7.4	6.7.7.5
6.7.7.6	6.7.7.7	6.7.7.8	6.7.7.9	6.7.8.1	6.7.8.2	6.7.8.3	6.7.8.4	6.7.8.5	6.7.8.6
6.7.8.7	6.7.8.8	6.7.8.9	6.7.9.1	6.7.9.2	6.7.9.3	6.7.9.4	6.7.9.5	6.7.9.6	6.7.9.7
6.7.9.8	6.7.9.9	6.8.1.1	6.8.1.2	6.8.1.3	6.8.1.4	6.8.1.5	6.8.1.6	6.8.1.7	6.8.1.8
6.8.1.9	6.8.2.1	6.8.2.2	6.8.2.3	6.8.2.4	6.8.2.5	6.8.2.6	6.8.2.7	6.8.2.8	6.8.2.9
6.8.3.1	6.8.3.2	6.8.3.3	6.8.3.4	6.8.3.5	6.8.3.6	6.8.3.7	6.8.3.8	6.8.3.9	6.8.4.
6.8.4.2	6.8.4.3	6.8.4.4	6.8.4.5	6.8.4.6	6.8.4.7	6.8.4.8	6.8.4.9	6.8.5.1	6.8.5.2
6.8.5.3	6.8.5.4	6.8.5.5	6.8.5.6	6.8.5.7	6.8.5.8	6.8.5.9	6.8.6.1	6.8.6.2	6.8.6.3
6.8.6.4	6.8.6.5	6.8.6.6	6.8.6.7	6.8.6.8	6.8.6.9	6.8.7.1	6.8.7.2	6.8.7.3	6.8.7.4
6.8.7.5	6.8.7.6	6.8.7.7	6.8.7.8	6.8.7.9	6.8.8.1	6.8.8.2	6.8.8.3	6.8.8.4	6.8.8.5
6.8.8.6	6.8.8.7	6.8.8.8	6.8.8.9	6.8.9.1	6.8.9.2	6.8.9.3	6.8.9.4	6.8.9.5	6.8.9.6
6.8.9.7	6.8.9.8	6.8.9.9	6.9.1.1	6.9.1.2	6.9.1.3	6.9.1.4	6.9.1.5	6.9.1.6	6.9.1.7
6.9.1.8	6.9.1.9	6.9.2.1	6.9.2.2	6.9.2.3	6.9.2.4	6.9.2.5	6.9.2.6	6.9.2.7	6.9.2.8
6.9.2.9	6.9.3.1	6.9.3.2	6.9.3.3	6.9.3.4	6.9.3.5	6.9.3.6	6.9.3.7	6.9.3.8	6.9.3.9
6.9.4.1	6.9.4.2	6.9.4.3	6.9.4.4	6.9.4.5	6.9.4.6	6.9.4.7	6.9.4.8	6.9.4.9	6.9.5.1
6.9.5.2	6.9.5.3	6.9.5.4	6.9.5.5	6.9.5.6	6.9.5.7	6.9.5.8	6.9.5.9	6.9.6.1	6.9.6.2
6.9.6.3	6.9.6.4	6.9.6.5	6.9.6.6	6.9.6.7	6.9.6.8	6.9.6.9	6.9.7.1	6.9.7.2	6.9.7.3
6.9.7.4	6.9.7.5	6.9.7.6	6.9.7.7	6.9.7.8	6.9.7.9	6.9.8.1	6.9.8.2	6.9.8.3	6.9.8.4
6.9.8.5	6.9.8.6	6.9.8.7	6.9.8.8	6.9.8.9	6.9.9.1	6.9.9.2	6.9.9.3	6.9.9.4	6.9.9.5
6.9.9.6	6.9.9.7	6.9.9.8	6.9.9.9	7.1.1.1	7.1.1.2	7.1.1.3	7.1.1.4	7.1.1.5	7.1.1.6
7.1.1.7	7.1.1.8	7.1.1.9	7.1.2.1	7.1.2.2	7.1.2.3	7.1.2.4	7.1.2.5	7.1.2.6	7.1.2.7
7.1.2.8	7.1.2.9	7.1.3.1	7.1.3.2	7.1.3.3	7.1.3.4	7.1.3.5	7.1.3.6	7.1.3.7	7.1.3.8
7.1.3.9	7.1.4.1	7.1.4.2	7.1.4.3	7.1.4.4	7.1.4.5	7.1.4.6	7.1.4.7	7.1.4.8	7.1.4.9
7.1.5.1	7.1.5.2	7.1.5.3	7.1.5.4	7.1.5.5	7.1.5.6	7.1.5.7	7.1.5.8	7.1.5.9	7.1.6.1
7.1.6.2	7.1.6.3	7.1.6.4	7.1.6.5	7.1.6.6	7.1.6.7	7.1.6.8	7.1.6.9	7.1.7.1	7.1.7.2
7.1.7.3	7.1.7.4	7.1.7.5	7.1.7.6	7.1.7.7	7.1.7.8	7.1.7.9	7.1.8.1	7.1.8.2	7.1.8.3
7.1.8.4	7.1.8.5	7.1.8.6	7.1.8.7	7.1.8.8	7.1.8.9	7.1.9.1	7.1.9.2	7.1.9.3	7.1.9.4
7.1.9.5	7.1.9.6	7.1.9.7	7.1.9.8	7.1.9.9	7.2.1.1	7.2.1.2	7.2.1.3	7.2.1.4	7.2.1.5
7.2.1.6	7.2.1.7	7.2.1.8	7.2.1.9	7.2.2.1	7.2.2.2	7.2.2.3	7.2.2.4	7.2.2.5	7.2.2.6
7.2.2.7	7.2.2.8	7.2.2.9	7.2.3.1	7.2.3.2	7.2.3.3	7.2.3.4	7.2.3.5	7.2.3.6	7.2.3.7

Table 1 - continued

	7.4.6.9	7.4.7.1	7.4.7.2	7.4.7.3	7.4.7.4	7.4.7.5	7.4.7.6	7.4.7.7	7.4.7.8	7.4.7.9
	7.4.8.1	7.4.8.2	7.4.8.3	7.4.8.4	7.4.8.5	7.4.8.6	7.4.8.7	7.4.8.8	7.4.8.9	7.4.9.1
	7.4.9.2	7.4.9.3	7.4.9.4	7.4.9.5	7.4.9.6	7.4.9.7	7.4.9.8	7.4.9.9	7.5.1.1	7.5.1.2
5	7.5.1.3	7.5.1.4	7.5.1.5	7.5.1.6	7.5.1.7	7.5.1.8	7.5.1.9	7.5.2.1	7.5.2.2	7.5.2.3
	7.5.2.4	7.5.2.5	7.5.2.6	7.5.2.7	7.5.2.8	7.5.2.9	7.5.3.1	7.5.3.2	7.5.3.3	7.5.3.4
	7.5.3.5	7.5.3.6	7.5.3.7	7.5.3.8	7.5.3.9	7.5.4.1	7.5.4.2	7.5.4.3	7.5.4.4	7.5.4.5
	7.5.4.6	7.5.4.7	7.5.4.8	7.5.4.9	7.5.5.1	7.5.5.2	7.5.5.3	7.5.5.4	7.5.5.5	7.5.5.6
	7.5.5.7	7.5.5.8	7.5.5.9	7.5.6.1	7.5.6.2	7.5.6.3	7.5.6.4	7.5.6.5	7.5.6.6	7.5.6.7
10	7.5.6.8	7.5.6.9	7.5.7.1	7.5.7.2	7.5.7.3	7.5.7.4	7.5.7.5	7.5.7.6	7.5.7.7	7.5.7.8
	7.5.7.9	7.5.8.1	7.5.8.2	7.5.8.3	7.5.8.4	7.5.8.5	7.5.8.6	7.5.8.7	7.5.8.8	7.5.8.9
	7.5.9.1	7.5.9.2	7.5.9.3	7.5.9.4	7.5.9.5	7.5.9.6	7.5.9.7	7.5.9.8	7.5.9.9	7.6.1.1
	7.6.1.2	7.6.1.3	7.6.1.4	7.6.1.5	7.6.1.6	7.6.1.7	7.6.1.8	7.6.1.9	7.6.2.1	7.6.2.2
	7.6.2.3	7.6.2.4	7.6.2.5	7.6.2.6	7.6.2.7	7.6.2.8	7.6.2.9	7.6.3.1	7.6.3.2	7.6.3.3
15	7.6.3.4	7.6.3.5	7.6.3.6	7.6.3.7	7.6.3.8	7.6.3.9	7.6.4.1	7.6.4.2	7.6.4.3	7.6.4.4
	7.6.4.5	7.6.4.6	7.6.4.7	7.6.4.8	7.6.4.9	7.6.5.1	7.6.5.2	7.6.5.3	7.6.5.4	7.6.5.5
	7.6.5.6	7.6.5.7	7.6.5.8	7.6.5.9	7.6.6.1	7.6.6.2	7.6.6.3	7.6.6.4	7.6.6.5	7.6.6.6
	7.6.6.7	7.6.6.8	7.6.6.9	7.6.7.1	7.6.7.2	7.6.7.3	7.6.7.4	7.6.7.5	7.6.7.6	7.6.7.7
	7.6.7.8	7.6.7.9	7.6.8.1	7.6.8.2	7.6.8.3	7.6.8.4	7.6.8.5	7.6.8.6	7.6.8.7	7.6.8.8
20	7.6.8.9	7.6.9.1	7.6.9.2	7.6.9.3	7.6.9.4	7.6.9.5	7.6.9.6	7.6.9.7	7.6.9.8	7.6.9.9
	7.7.1.1	7.7.1.2	7.7.1.3	7.7.1.4	7.7.1.5	7.7.1.6	7.7.1.7	7.7.1.8	7.7.1.9	7.7.2.1
	7.7.2.2	7.7.2.3	7.7.2.4	7.7.2.5	7.7.2.6	7.7.2.7	7.7.2.B	7.7.2.9	7.7.3.1	7.7.3.2
	7.7.3.3	7.7.3.4	7.7.3.5	7.7.3.6	7.7.3.7	7.7.3.8	7.7.3.9	7.7.4.1	7.7.4.2	7.7.4.3
	7.7.4.4	7.7.4.5	7.7.4.6	7.7.4.7	7.7.4.8	7.7.4.9	7.7.5.1	7.7.5.2	7.7.5.3	7.7.5.4
25	7.7.5.5	7.7.5.6	7.7.5.7	7.7.5.8	7.7.5.9	7.7.6.1	7.7.6.2	7.7.6.3	7.7.6.4	7.7.6.5
	7.7.6.6	7.7.6.7	7.7.6.8	7.7.6.9	7.7.7.1	7.7.7.2	7.7.7.3	7.7.7.4	7.7.7.5	7.7.7.6
	7.7.7.7	7.7.7.8	7.7.7.9	7.7.8.1	7.7.8.2	7.7.8.3	7.7.8.4	7.7.8.5	7.7.8.6	7.7.8.7
	7.7.8.8	7.7.8.9	7.7.9.1	7.7.9.2	7.7.9.3	7.7.9.4	7.7.9.5	7.7.9.6	7.7.9.7	7.7.9.8
	7.7.9.9	7.8.1.1	7.8.1.2	7.8.1.3	7.8.1.4	7.8.1.5	7.8.1.6	7.8.1.7	7.8.1.8	7.8.1.9
30	7.8.2.1	7.8.2.2	7.8.2.3	7.8.2.4	7.8.2.5	7.8.2.6	7.8.2.7	7.8.2.8	7.8.2.9	7.8.3.1
	7.8.3.2	7.8.3.3	7.8.3.4	7.8.3.5	7.8.3.6	7.8.3.7	7.8.3.8	7.8.3.9	7.8.4.1	7.8.4.2
	7.8.4.3	7.8.4.4	7.8.4.5	7.8.4.6	7.8.4.7	7.8.4.8	7.8.4.9	7.8.5.1	7.8.5.2	7.8.5.3
	7.8.5.4	7.8.5.5	7.8.5.6	7.8.5.7	7.8.5.8	7.8.5.9	7.8.6.1	7.8.6.2	7.8.6.3	7.8.6.4
	7.8.6.5	7.8.6.6	7.8.6.7	7.8.6.8	7.8.6.9	7.8.7.1	7.8.7.2	7.8.7.3	7.8.7.4	7.8.7.5
35	7.8.7.6	7.8.7.7	7.8.7.8	7.8.7.9	7.8.8.1	7.8.8.2	7.8.8.3	7.8.8.4	7.8.8.5	7.8.8.6
	7.8.8.7	7.8.8.8	7.8.8.9	7.8.9.1	7.8.9.2	7.8.9.3	7.8.9.4	7.8.9.5	7.8.9.6	7.8.9.7
	7.8.9.8	7.8.9.9	7.9.1.1	7.9.1.2	7.9.1.3	7.9.1.4	7.9.1.5	7.9.1.6	7.9.1.7	7.9.1.8
	7.9.1.9	7.9.2.1	7.9.2.2	7.9.2.3	7.9.2.4	7.9.2.5	7.9.2.6	7.9.2.7	7.9.2.8	7.9.2.9
	7.9.3.1	7.9.3.2	7.9.3.3	7.9.3.4	7.9.3.5	7.9.3.6	7.9.3.7	7.9.3.8	7.9.3.9	7.9.4.1
40	7.9.4.2	7.9.4.3	7.9.4.4	7.9.4.5	7.9.4.6	7.9.4.7	7.9.4.8	7.9.4.9	7.9.5.1	7.9.5.2
	7.9.5.3	7.9.5.4	7.9.5.5	7.9.5.6	7.9.5.7	7.9.5.8	7.9.5.9	7.9.6.1	7.9.6.2	7.9.6.3
	7.9.6.4	7.9.6.5	7.9.6.6	7.9.6.7	7.9.6.8	7.9.6.9	7.9.7.1	7.9.7.2	7.9.7.3	7.9.7.4
	7.9.7.5	7.9.7.6	7.9.7.7	7.9.7.8	7.9.7.9	7.9.8.1	7.9.8.2	7.9.8.3	7.9.8.4	7.9.8.5
	7.9.8.6	7.9.8.7	7.9.8.8	7.9.8.9	7.9.9.1	7.9.9.2	7.9.9.3	7.9.9.4	7.9.9.5	7.9.9.6
45	7.9.9.7	7.9.9.8	7.9.9.9	8.1.1.1	8.1.1.2	8.1.1.3	8.1.1.4	8.1.1.5	8.1.1.6	8.1.1.7
	8.1.1.8	8.1.1.9	8.1.2.1	8.1.2.2	8.1.2.3	8.1.2.4	8.1.2.5	8.1.2.6	8.1.2.7	8.1.2.8
	8.1.2.9	8.1.3.1	8.1.3.2	8.1.3.3	8.1.3.4	8.1.3.5	8.1.3.6	8.1.3.7	8.1.3.8	8.1.3.9
	8.1.4.1	8.1.4.2	8.1.4.3	8.1.4.4	8.1.4.5	8.1.4.6	8.1.4.7	8.1.4.8	8.1.4.9	8.1.5.1
	8.1.5.2	8.1.5.3	8.1.5.4	8.1.5.5	8.1.5.6	8.1.5.7	8.1.5.8	8.1.5.9	8.1.6.1	8.1.6.2
50	8.1.6.3	8.1.6.4	8.1.6.5	8.1.6.6	8.1.6.7	8.1.6.8	8.1.6.9	8.1.7.1	8.1.7.2	8.1.7.3
	8.1.7.4	8.1.7.5	8.1.7.6	8.1.7.7	8.1.7.8	8.1.7.9	8.1.8.1	8.1.8.2	8.1.8.3	8.1.8.4
	8.1.8.5	8.1.8.6	8.1.8.7	8.1.8.8	8.1.8.9	8.1.9.1	8.1.9.2	8.1.9.3	8.1.9.4	8.1.9.5
	8.1.9.6	8.1.9.7	8.1.9.8	8.1.9.9	8.2.1.1	8.2.1.2	8.2.1.3	8.2.1.4	8.2.1.5	8.2.1.6

00518501-030300

Table 1 - continued

	8.2.1.7	8.2.1.8	8.2.1.9	8.2.2.1	8.2.2.2	8.2.2.3	8.2.2.4	8.2.2.5	8.2.2.6	8.2.2.7
	8.2.2.8	8.2.2.9	8.2.3.1	8.2.3.2	8.2.3.3	8.2.3.4	8.2.3.5	8.2.3.6	8.2.3.7	8.2.3.8
	8.2.3.9	8.2.4.1	8.2.4.2	8.2.4.3	8.2.4.4	8.2.4.5	8.2.4.6	8.2.4.7	8.2.4.8	8.2.4.9
5	8.2.5.1	8.2.5.2	8.2.5.3	8.2.5.4	8.2.5.5	8.2.5.6	8.2.5.7	8.2.5.8	8.2.5.9	8.2.6.1
	8.2.6.2	8.2.6.3	8.2.6.4	8.2.6.5	8.2.6.6	8.2.6.7	8.2.6.8	8.2.6.9	8.2.7.1	8.2.7.2
	8.2.7.3	8.2.7.4	8.2.7.5	8.2.7.6	8.2.7.7	8.2.7.8	8.2.7.9	8.2.8.1	8.2.8.2	8.2.8.3
	8.2.8.4	8.2.8.5	8.2.8.6	8.2.8.7	8.2.8.8	8.2.8.9	8.2.9.1	8.2.9.2	8.2.9.3	8.2.9.4
	8.2.9.5	8.2.9.6	8.2.9.7	8.2.9.8	8.2.9.9	8.3.1.1	8.3.1.2	8.3.1.3	8.3.1.4	8.3.1.5
10	8.3.1.6	8.3.1.7	8.3.1.8	8.3.1.9	8.3.2.1	8.3.2.2	8.3.2.3	8.3.2.4	8.3.2.5	8.3.2.6
	8.3.2.7	8.3.2.8	8.3.2.9	8.3.3.1	8.3.3.2	8.3.3.3	8.3.3.4	8.3.3.5	8.3.3.6	8.3.3.7
	8.3.3.8	8.3.3.9	8.3.4.1	8.3.4.2	8.3.4.3	8.3.4.4	8.3.4.5	8.3.4.6	8.3.4.7	8.3.4.8
	8.3.4.9	8.3.5.1	8.3.5.2	8.3.5.3	8.3.5.4	8.3.5.5	8.3.5.6	8.3.5.7	8.3.5.8	8.3.5.9
	8.3.6.1	8.3.6.2	8.3.6.3	8.3.6.4	8.3.6.5	8.3.6.6	8.3.6.7	8.3.6.8	8.3.6.9	8.3.7.1
15	8.3.7.2	8.3.7.3	8.3.7.4	8.3.7.5	8.3.7.6	8.3.7.7	8.3.7.8	8.3.7.9	8.3.8.1	8.3.8.2
	8.3.8.3	8.3.8.4	8.3.8.5	8.3.8.6	8.3.8.7	8.3.8.8	8.3.8.9	8.3.9.1	8.3.9.2	8.3.9.3
	8.3.9.4	8.3.9.5	8.3.9.6	8.3.9.7	8.3.9.8	8.3.9.9	8.4.1.1	8.4.1.2	8.4.1.3	8.4.1.4
	8.4.1.5	8.4.1.6	8.4.1.7	8.4.1.8	8.4.1.9	8.4.2.1	8.4.2.2	8.4.2.3	8.4.2.4	8.4.2.5
	8.4.2.6	8.4.2.7	8.4.2.8	8.4.2.9	8.4.3.1	8.4.3.2	8.4.3.3	8.4.3.4	8.4.3.5	8.4.3.6
20	8.4.3.7	8.4.3.8	8.4.3.9	8.4.4.1	8.4.4.2	8.4.4.3	8.4.4.4	8.4.4.5	8.4.4.6	8.4.4.7
	8.4.4.8	8.4.4.9	8.4.5.1	8.4.5.2	8.4.5.3	8.4.5.4	8.4.5.5	8.4.5.6	8.4.5.7	8.4.5.8
	8.4.5.9	8.4.6.1	8.4.6.2	8.4.6.3	8.4.6.4	8.4.6.5	8.4.6.6	8.4.6.7	8.4.6.8	8.4.6.9
	8.4.7.1	8.4.7.2	8.4.7.3	8.4.7.4	8.4.7.5	8.4.7.6	8.4.7.7	8.4.7.8	8.4.7.9	8.4.8.1
	8.4.8.2	8.4.8.3	8.4.8.4	8.4.8.5	8.4.8.6	8.4.8.7	8.4.8.8	8.4.8.9	8.4.9.1	8.4.9.2
25	8.4.9.3	8.4.9.4	8.4.9.5	8.4.9.6	8.4.9.7	8.4.9.8	8.4.9.9	8.5.1.1	8.5.1.2	8.5.1.3
	8.5.1.4	8.5.1.5	8.5.1.6	8.5.1.7	8.5.1.8	8.5.1.9	8.5.2.1	8.5.2.2	8.5.2.3	8.5.2.4
	8.5.2.5	8.5.2.6	8.5.2.7	8.5.2.8	8.5.2.9	8.5.3.1	8.5.3.2	8.5.3.3	8.5.3.4	8.5.3.5
	8.5.3.6	8.5.3.7	8.5.3.8	8.5.3.9	8.5.4.1	8.5.4.2	8.5.4.3	8.5.4.4	8.5.4.5	8.5.4.6
	8.5.4.7	8.5.4.8	8.5.4.9	8.5.5.1	8.5.5.2	8.5.5.3	8.5.5.4	8.5.5.5	8.5.5.6	8.5.5.7
30	8.5.5.8	8.5.5.9	8.5.6.1	8.5.6.2	8.5.6.3	8.5.6.4	8.5.6.5	8.5.6.6	8.5.6.7	8.5.6.8
	8.5.6.9	8.5.7.1	8.5.7.2	8.5.7.3	8.5.7.4	8.5.7.5	8.5.7.6	8.5.7.7	8.5.7.8	8.5.7.9
	8.5.8.1	8.5.8.2	8.5.8.3	8.5.8.4	8.5.8.5	8.5.8.6	8.5.8.7	8.5.8.8	8.5.8.9	8.5.9.1
	8.5.9.2	8.5.9.3	8.5.9.4	8.5.9.5	8.5.9.6	8.5.9.7	8.5.9.8	8.5.9.9	8.6.1.1	8.6.1.2
	8.6.1.3	8.6.1.4	8.6.1.5	8.6.1.6	8.6.1.7	8.6.1.8	8.6.1.9	8.6.2.1	8.6.2.2	8.6.2.3
35	8.6.2.4	8.6.2.5	8.6.2.6	8.6.2.7	8.6.2.8	8.6.2.9	8.6.3.1	8.6.3.2	8.6.3.3	8.6.3.4
	8.6.3.5	8.6.3.6	8.6.3.7	8.6.3.8	8.6.3.9	8.6.4.1	8.6.4.2	8.6.4.3	8.6.4.4	8.6.4.5
	8.6.4.6	8.6.4.7	8.6.4.8	8.6.4.9	8.6.5.1	8.6.5.2	8.6.5.3	8.6.5.4	8.6.5.5	8.6.5.6
	8.6.5.7	8.6.5.8	8.6.5.9	8.6.6.1	8.6.6.2	8.6.6.3	8.6.6.4	8.6.6.5	8.6.6.6	8.6.6.7
	8.6.6.8	8.6.6.9	8.6.7.1	8.6.7.2	8.6.7.3	8.6.7.4	8.6.7.5	8.6.7.6	8.6.7.7	8.6.7.8
40	8.6.7.9	8.6.8.1	8.6.8.2	8.6.8.3	8.6.8.4	8.6.8.5	8.6.8.6	8.6.8.7	8.6.8.8	8.6.8.9
	8.6.9.1	8.6.9.2	8.6.9.3	8.6.9.4	8.6.9.5	8.6.9.6	8.6.9.7	8.6.9.8	8.6.9.9	8.7.1.1
	8.7.1.2	8.7.1.3	8.7.1.4	8.7.1.5	8.7.1.6	8.7.1.7	8.7.1.8	8.7.1.9	8.7.2.1	8.7.2.2
	8.7.2.3	8.7.2.4	8.7.2.5	8.7.2.6	8.7.2.7	8.7.2.8	8.7.2.9	8.7.3.1	8.7.3.2	8.7.3.3
	8.7.3.4	8.7.3.5	8.7.3.6	8.7.3.7	8.7.3.8	8.7.3.9	8.7.4.1	8.7.4.2	8.7.4.3	8.7.4.4
45	8.7.4.5	8.7.4.6	8.7.4.7	8.7.4.8	8.7.4.9	8.7.5.1	8.7.5.2	8.7.5.3	8.7.5.4	8.7.5.5
	8.7.5.6	8.7.5.7	8.7.5.8	8.7.5.9	8.7.6.1	8.7.6.2	8.7.6.3	8.7.6.4	8.7.6.5	8.7.6.6
	8.7.6.7	8.7.6.8	8.7.6.9	8.7.7.1	8.7.7.2	8.7.7.3	8.7.7.4	8.7.7.5	8.7.7.6	8.7.7.7
	8.7.7.8	8.7.7.9	8.7.8.1	8.7.8.2	8.7.8.3	8.7.8.4	8.7.8.5	8.7.8.6	8.7.8.7	8.7.8.8
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	8.8.4.4	8.8.4.5	8.8.4.6	8.8.4.7	8.8.4.8	8.8.4.9	8.8.5.1	8.8.5.2	8.8.5.3	8.8.5.4

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8.8.7.7	8.8.7.8	8.8.7.9	8.8.8.1	8.8.8.2	8.8.8.3	8.8.8.4	8.8.8.5	8.8.8.6	8.8.8.7
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8.9.3.2	8.9.3.3	8.9.3.4	8.9.3.5	8.9.3.6	8.9.3.7	8.9.3.8	8.9.3.9	8.9.4.1	8.9.4.2
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8.9.7.6	8.9.7.7	8.9.7.8	8.9.7.9	8.9.8.1	8.9.8.2	8.9.8.3	8.9.8.4	8.9.8.5	8.9.8.6
8.9.8.7	8.9.8.8	8.9.8.9	8.9.9.1	8.9.9.2	8.9.9.3	8.9.9.4	8.9.9.5	8.9.9.6	8.9.9.7
8.9.9.8	8.9.9.9	9.1.1.1	9.1.1.2	9.1.1.3	9.1.1.4	9.1.1.5	9.1.1.6	9.1.1.7	9.1.1.8
9.1.1.9	9.1.2.1	9.1.2.2	9.1.2.3	9.1.2.4	9.1.2.5	9.1.2.6	9.1.2.7	9.1.2.8	9.1.2.9
9.1.3.1	9.1.3.2	9.1.3.3	9.1.3.4	9.1.3.5	9.1.3.6	9.1.3.7	9.1.3.8	9.1.3.9	9.1.4.1
9.1.4.2	9.1.4.3	9.1.4.4	9.1.4.5	9.1.4.6	9.1.4.7	9.1.4.8	9.1.4.9	9.1.5.1	9.1.5.2
9.1.5.3	9.1.5.4	9.1.5.5	9.1.5.6	9.1.5.7	9.1.5.8	9.1.5.9	9.1.6.1	9.1.6.2	9.1.6.3
9.1.6.4	9.1.6.5	9.1.6.6	9.1.6.7	9.1.6.8	9.1.6.9	9.1.7.1	9.1.7.2	9.1.7.3	9.1.7.4
9.1.7.5	9.1.7.6	9.1.7.7	9.1.7.8	9.1.7.9	9.1.8.1	9.1.8.2	9.1.8.3	9.1.8.4	9.1.8.5
9.1.8.6	9.1.8.7	9.1.8.8	9.1.8.9	9.1.9.1	9.1.9.2	9.1.9.3	9.1.9.4	9.1.9.5	9.1.9.6
9.1.9.7	9.1.9.8	9.1.9.9	9.2.1.1	9.2.1.2	9.2.1.3	9.2.1.4	9.2.1.5	9.2.1.6	9.2.1.7
9.2.1.8	9.2.1.9	9.2.2.1	9.2.2.2	9.2.2.3	9.2.2.4	9.2.2.5	9.2.2.6	9.2.2.7	9.2.2.8
9.2.2.9	9.2.3.1	9.2.3.2	9.2.3.3	9.2.3.4	9.2.3.5	9.2.3.6	9.2.3.7	9.2.3.8	9.2.3.9
9.2.4.1	9.2.4.2	9.2.4.3	9.2.4.4	9.2.4.5	9.2.4.6	9.2.4.7	9.2.4.8	9.2.4.9	9.2.5.1
9.2.5.2	9.2.5.3	9.2.5.4	9.2.5.5	9.2.5.6	9.2.5.7	9.2.5.8	9.2.5.9	9.2.6.1	9.2.6.2
9.2.6.3	9.2.6.4	9.2.6.5	9.2.6.6	9.2.6.7	9.2.6.8	9.2.6.9	9.2.7.1	9.2.7.2	9.2.7.3
9.2.7.4	9.2.7.5	9.2.7.6	9.2.7.7	9.2.7.8	9.2.7.9	9.2.8.1	9.2.8.2	9.2.8.3	9.2.8.4
9.2.8.5	9.2.8.6	9.2.8.7	9.2.8.8	9.2.8.9	9.2.9.1	9.2.9.2	9.2.9.3	9.2.9.4	9.2.9.5
9.2.9.6	9.2.9.7	9.2.9.8	9.2.9.9	9.3.1.1	9.3.1.2	9.3.1.3	9.3.1.4	9.3.1.5	9.3.1.6
9.3.1.7	9.3.1.8	9.3.1.9	9.3.2.1	9.3.2.2	9.3.2.3	9.3.2.4	9.3.2.5	9.3.2.6	9.3.2.7
9.3.2.8	9.3.2.9	9.3.3.1	9.3.3.2	9.3.3.3	9.3.3.4	9.3.3.5	9.3.3.6	9.3.3.7	9.3.3.8
9.3.3.9	9.3.4.1	9.3.4.2	9.3.4.3	9.3.4.4	9.3.4.5	9.3.4.6	9.3.4.7	9.3.4.8	9.3.4.9
9.3.5.1	9.3.5.2	9.3.5.3	9.3.5.4	9.3.5.5	9.3.5.6	9.3.5.7	9.3.5.8	9.3.5.9	9.3.6.1

Table 1 - continued

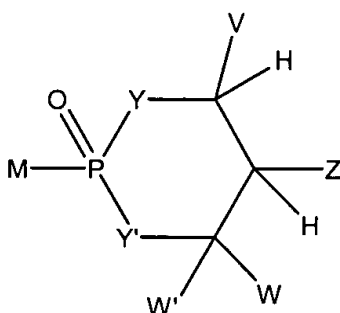
	9.5.9.3	9.5.9.4	9.5.9.5	9.5.9.6	9.5.9.7	9.5.9.8	9.5.9.9	9.6.1.1	9.6.1.2	9.6.1.3
	9.6.1.4	9.6.1.5	9.6.1.6	9.6.1.7	9.6.1.8	9.6.1.9	9.6.2.1	9.6.2.2	9.6.2.3	9.6.2.4
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	9.6.8.1	9.6.8.2	9.6.8.3	9.6.8.4	9.6.8.5	9.6.8.6	9.6.8.7	9.6.8.8	9.6.8.9	9.6.9.1
10	9.6.9.2	9.6.9.3	9.6.9.4	9.6.9.5	9.6.9.6	9.6.9.7	9.6.9.8	9.6.9.9	9.7.1.1	9.7.1.2
	9.7.1.3	9.7.1.4	9.7.1.5	9.7.1.6	9.7.1.7	9.7.1.8	9.7.1.9	9.7.2.1	9.7.2.2	9.7.2.3
	9.7.2.4	9.7.2.5	9.7.2.6	9.7.2.7	9.7.2.8	9.7.2.9	9.7.3.1	9.7.3.2	9.7.3.3	9.7.3.4
	9.7.3.5	9.7.3.6	9.7.3.7	9.7.3.8	9.7.3.9	9.7.4.1	9.7.4.2	9.7.4.3	9.7.4.4	9.7.4.5
	9.7.4.6	9.7.4.7	9.7.4.8	9.7.4.9	9.7.5.1	9.7.5.2	9.7.5.3	9.7.5.4	9.7.5.5	9.7.5.6
15	9.7.5.7	9.7.5.8	9.7.5.9	9.7.6.1	9.7.6.2	9.7.6.3	9.7.6.4	9.7.6.5	9.7.6.6	9.7.6.7
	9.7.6.8	9.7.6.9	9.7.7.1	9.7.7.2	9.7.7.3	9.7.7.4	9.7.7.5	9.7.7.6	9.7.7.7	9.7.7.8
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	9.7.9.1	9.7.9.2	9.7.9.3	9.7.9.4	9.7.9.5	9.7.9.6	9.7.9.7	9.7.9.8	9.7.9.9	9.8.1.1
	9.8.1.2	9.8.1.3	9.8.1.4	9.8.1.5	9.8.1.6	9.8.1.7	9.8.1.8	9.8.1.9	9.8.2.1	9.8.2.2
20	9.8.2.3	9.8.2.4	9.8.2.5	9.8.2.6	9.8.2.7	9.8.2.8	9.8.2.9	9.8.3.1	9.8.3.2	9.8.3.3
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	9.8.5.6	9.8.5.7	9.8.5.8	9.8.5.9	9.8.6.1	9.8.6.2	9.8.6.3	9.8.6.4	9.8.6.5	9.8.6.6
	9.8.6.7	9.8.6.8	9.8.6.9	9.8.7.1	9.8.7.2	9.8.7.3	9.8.7.4	9.8.7.5	9.8.7.6	9.8.7.7
25	9.8.7.8	9.8.7.9	9.8.8.1	9.8.8.2	9.8.8.3	9.8.8.4	9.8.8.5	9.8.8.6	9.8.8.7	9.8.8.8
	9.8.8.9	9.8.9.1	9.8.9.2	9.8.9.3	9.8.9.4	9.8.9.5	9.8.9.6	9.8.9.7	9.8.9.8	9.8.9.9
	9.9.1.1	9.9.1.2	9.9.1.3	9.9.1.4	9.9.1.5	9.9.1.6	9.9.1.7	9.9.1.8	9.9.1.9	9.9.2.1
	9.9.2.2	9.9.2.3	9.9.2.4	9.9.2.5	9.9.2.6	9.9.2.7	9.9.2.8	9.9.2.9	9.9.3.1	9.9.3.2
	9.9.3.3	9.9.3.4	9.9.3.5	9.9.3.6	9.9.3.7	9.9.3.8	9.9.3.9	9.9.4.1	9.9.4.2	9.9.4.3
30	9.9.4.4	9.9.4.5	9.9.4.6	9.9.4.7	9.9.4.8	9.9.4.9	9.9.5.1	9.9.5.2	9.9.5.3	9.9.5.4
	9.9.5.5	9.9.5.6	9.9.5.7	9.9.5.8	9.9.5.9	9.9.6.1	9.9.6.2	9.9.6.3	9.9.6.4	9.9.6.5
	9.9.6.6	9.9.6.7	9.9.6.8	9.9.6.9	9.9.7.1	9.9.7.2	9.9.7.3	9.9.7.4	9.9.7.5	9.9.7.6
	9.9.7.7	9.9.7.8	9.9.7.9	9.9.8.1	9.9.8.2	9.9.8.3	9.9.8.4	9.9.8.5	9.9.8.6	9.9.8.7
	9.9.8.8	9.9.8.9	9.9.9.1	9.9.9.2	9.9.9.3	9.9.9.4	9.9.9.5	9.9.9.6	9.9.9.7	9.9.9.8
35	9.9.9.9									

09518501-030300

Another group of preferred compounds are named in Table 2 and designated by numbers assigned to the variables of formula I using the following convention:

$M^1.Y/Y'.V/Z/W$. The compounds are shown without depiction of stereochemistry since the compounds are biologically active as the diastereomeric mixture or as a single stereoisomer. M^1 is a variable that represents compounds of the formula M-H which have a specific hydroxyl group that is phosphorylated with a group $P(O)[Y-CH(V)CH(Z)CH(W)-Y']$ to make compounds of formula I or M^1 is a variable that represents phosphonic acids of the formula $M-PO_3^{=}$ which are transformed to compounds of formula I by replacing two oxygens in the $PO_3^{=}$ group with $Y-CH(V)CH(Z)CH(W)-Y'$.

The structures for variable M^1 are the same as described above.



Variable Y/Y'

- | | | |
|----|----|---|
| | 1) | Y= NH; Y'= oxygen |
| 15 | 2) | Y= oxygen; Y'=NH |
| | 3) | Y=NH; Y' = NH |
| | 4) | Y=N-CH ₃ ; Y'= oxygen |
| | 5) | Y= oxygen; Y'=NCH ₃ |
| | 6) | Y=N-CH ₂ CH ₃ ; Y' = oxygen |
| 20 | 7) | Y = N-phenyl; Y' = oxygen |
| | 8) | Y = Ni-propyl; Y' = oxygen |
| | 9) | Y = oxygen; Y'=N-CH ₂ CH ₃ |

Variable V/Z/W: Group V/Z/W1

- | | | |
|----|----|---|
| | 1) | V= phenyl; Z = methyl; W = hydrogen |
| | 2) | V= 3,5-dichlorophenyl; Z = methyl; W = hydrogen |
| | 3) | V= 4-pyridyl; Z = methyl; W = hydrogen |
| 5 | 4) | V= phenyl; Z = methoxy; W = hydrogen |
| | 5) | V= 3,5-dichlorophenyl; Z = methoxy; W = hydrogen |
| | 6) | V= 4-pyridyl; Z = methoxy; W = hydrogen |
| | 7) | V= phenyl; Z = hydrogen; W = phenyl |
| | 8) | V= 3,5-dichlorophenyl; Z = hydrogen; W = 3,5-dichlorophenyl |
| 10 | 9) | V= 4-pyridyl; Z = hydrogen; W = 4-pyridyl |

Variable V/Z/W: Group V/Z/W2

- | | | |
|----|----|--|
| | 1) | V= phenyl; Z = NHAc; W = hydrogen |
| | 2) | V= 3,5-dichlorophenyl; Z = NHAc; W = hydrogen |
| 15 | 3) | V= 4-pyridyl; Z = NHAc; W = hydrogen |
| | 4) | V= phenyl; Z = hydrogen; W = methyl |
| | 5) | V= 3,5-dichlorophenyl; Z = hydrogen; W = methyl |
| | 6) | V= 4-pyridyl; Z = hydrogen; W = methyl |
| | 7) | V= phenyl; Z = hydroxy; W = hydrogen |
| 20 | 8) | V= 3,5-dichlorophenyl; Z = hydroxy; W = hydrogen |
| | 9) | V= 4-pyridyl; Z = hydroxy; W = hydrogen |

Variable V/Z/W: Group V/Z/W3

- | | | |
|----|----|---|
| | 1) | V=hydrogen; Z=CH ₂ OH; W=hydrogen |
| 25 | 2) | V=hydrogen; Z=CH ₂ OC(O)CH ₃ ; W=hydrogen |
| | 3) | V=hydrogen; Z=CH ₂ OC(O)OCH ₃ ; W=hydrogen |
| | 4) | V=methyl; Z=CH ₂ OH; W=hydrogen |
| | 5) | V=methyl; Z=CH ₂ OC(O)CH ₃ ; W=hydrogen |
| | 6) | V=methyl; Z=CH ₂ OC(O)OCH ₃ ; W=hydrogen |
| 30 | 7) | Z= hydrogen; V and W = -CH ₂ -CH(OH)CH ₂ - |
| | 8) | Z= hydrogen; V and W = -CH ₂ -CH(OAc)CH ₂ - |
| | 9) | Z= hydrogen; V and W = -CH ₂ -CH(OCO ₂ CH ₂ CH ₃)CH ₂ - |

5

10

15

Table 2

5	1.1.1	1.1.2	1.1.3	1.1.4	1.1.5	1.1.6	1.1.7	1.1.8	1.1.9	1.2.1	1.2.2	1.2.3
	1.2.4	1.2.5	1.2.6	1.2.7	1.2.8	1.2.9	1.3.1	1.3.2	1.3.3	1.3.4	1.3.5	1.3.6
	1.3.7	1.3.8	1.3.9	1.4.1	1.4.2	1.4.3	1.4.4	1.4.5	1.4.6	1.4.7	1.4.8	1.4.9
	1.5.1	1.5.2	1.5.3	1.5.4	1.5.5	1.5.6	1.5.7	1.5.8	1.5.9	1.6.1	1.6.2	1.6.3
	1.6.4	1.6.5	1.6.6	1.6.7	1.6.8	1.6.9	1.7.1	1.7.2	1.7.3	1.7.4	1.7.5	1.7.6
	1.7.7	1.7.8	1.7.9	1.8.1	1.8.2	1.8.3	1.8.4	1.8.5	1.8.6	1.8.7	1.8.8	1.8.9
10	1.9.1	1.9.2	1.9.3	1.9.4	1.9.5	1.9.6	1.9.7	1.9.8	1.9.9	2.1.1	2.1.2	2.1.3
	2.1.4	2.1.5	2.1.6	2.1.7	2.1.8	2.1.9	2.2.1	2.2.2	2.2.3	2.2.4	2.2.5	2.2.6
	2.2.7	2.2.8	2.2.9	2.3.1	2.3.2	2.3.3	2.3.4	2.3.5	2.3.6	2.3.7	2.3.8	2.3.9
	2.4.1	2.4.2	2.4.3	2.4.4	2.4.5	2.4.6	2.4.7	2.4.8	2.4.9	2.5.1	2.5.2	2.5.3
	2.5.4	2.5.5	2.5.6	2.5.7	2.5.8	2.5.9	2.6.1	2.6.2	2.6.3	2.6.4	2.6.5	2.6.6
	2.6.7	2.6.8	2.6.9	2.7.1	2.7.2	2.7.3	2.7.4	2.7.5	2.7.6	2.7.7	2.7.8	2.7.9
15	2.8.1	2.8.2	2.8.3	2.8.4	2.8.5	2.8.6	2.8.7	2.8.8	2.8.9	2.9.1	2.9.2	2.9.3
	2.9.4	2.9.5	2.9.6	2.9.7	2.9.8	2.9.9	3.1.1	3.1.2	3.1.3	3.1.4	3.1.5	3.1.6
	3.1.7	3.1.8	3.1.9	3.2.1	3.2.2	3.2.3	3.2.4	3.2.5	3.2.6	3.2.7	3.2.8	3.2.9
	3.3.1	3.3.2	3.3.3	3.3.4	3.3.5	3.3.6	3.3.7	3.3.8	3.3.9	3.4.1	3.4.2	3.4.3
	3.4.4	3.4.5	3.4.6	3.4.7	3.4.8	3.4.9	3.5.1	3.5.2	3.5.3	3.5.4	3.5.5	3.5.6
	3.5.7	3.5.8	3.5.9	3.6.1	3.6.2	3.6.3	3.6.4	3.6.5	3.6.6	3.6.7	3.6.8	3.6.9
20	3.7.1	3.7.2	3.7.3	3.7.4	3.7.5	3.7.6	3.7.7	3.7.8	3.7.9	3.8.1	3.8.2	3.8.3
	3.8.4	3.8.5	3.8.6	3.8.7	3.8.8	3.8.9	3.9.1	3.9.2	3.9.3	3.9.4	3.9.5	3.9.6
	3.9.7	3.9.8	3.9.9	4.1.1	4.1.2	4.1.3	4.1.4	4.1.5	4.1.6	4.1.7	4.1.8	4.1.9
	4.2.1	4.2.2	4.2.3	4.2.4	4.2.5	4.2.6	4.2.7	4.2.8	4.2.9	4.3.1	4.3.2	4.3.3
	4.3.4	4.3.5	4.3.6	4.3.7	4.3.8	4.3.9	4.4.1	4.4.2	4.4.3	4.4.4	4.4.5	4.4.6
	4.4.7	4.4.8	4.4.9	4.5.1	4.5.2	4.5.3	4.5.4	4.5.5	4.5.6	4.5.7	4.5.8	4.5.9
25	4.6.1	4.6.2	4.6.3	4.6.4	4.6.5	4.6.6	4.6.7	4.6.8	4.6.9	4.7.1	4.7.2	4.7.3
	4.7.4	4.7.5	4.7.6	4.7.7	4.7.8	4.7.9	4.8.1	4.8.2	4.8.3	4.8.4	4.8.5	4.8.6
	4.8.7	4.8.8	4.8.9	4.9.1	4.9.2	4.9.3	4.9.4	4.9.5	4.9.6	4.9.7	4.9.8	4.9.9
	5.1.1	5.1.2	5.1.3	5.1.4	5.1.5	5.1.6	5.1.7	5.1.8	5.1.9	5.2.1	5.2.2	5.2.3
	5.2.4	5.2.5	5.2.6	5.2.7	5.2.8	5.2.9	5.3.1	5.3.2	5.3.3	5.3.4	5.3.5	5.3.6
	5.3.7	5.3.8	5.3.9	5.4.1	5.4.2	5.4.3	5.4.4	5.4.5	5.4.6	5.4.7	5.4.8	5.4.9
30	5.5.1	5.5.2	5.5.3	5.5.4	5.5.5	5.5.6	5.5.7	5.5.8	5.5.9	5.6.1	5.6.2	5.6.3
	5.6.4	5.6.5	5.6.6	5.6.7	5.6.8	5.6.9	5.7.1	5.7.2	5.7.3	5.7.4	5.7.5	5.7.6
	5.7.7	5.7.8	5.7.9	5.8.1	5.8.2	5.8.3	5.8.4	5.8.5	5.8.6	5.8.7	5.8.8	5.8.9
	5.9.1	5.9.2	5.9.3	5.9.4	5.9.5	5.9.6	5.9.7	5.9.8	5.9.9	6.1.1	6.1.2	6.1.3
	6.1.4	6.1.5	6.1.6	6.1.7	6.1.8	6.1.9	6.2.1	6.2.2	6.2.3	6.2.4	6.2.5	6.2.6
	6.2.7	6.2.8	6.2.9	6.3.1	6.3.2	6.3.3	6.3.4	6.3.5	6.3.6	6.3.7	6.3.8	6.3.9
35	6.4.1	6.4.2	6.4.3	6.4.4	6.4.5	6.4.6	6.4.7	6.4.8	6.4.9	6.5.1	6.5.2	6.5.3
	6.5.4	6.5.5	6.5.6	6.5.7	6.5.8	6.5.9	6.6.1	6.6.2	6.6.3	6.6.4	6.6.5	6.6.6
	6.6.7	6.6.8	6.6.9	6.7.1	6.7.2	6.7.3	6.7.4	6.7.5	6.7.6	6.7.7	6.7.8	6.7.9
	6.8.1	6.8.2	6.8.3	6.8.4	6.8.5	6.8.6	6.8.7	6.8.8	6.8.9	6.9.1	6.9.2	6.9.3
	6.9.4	6.9.5	6.9.6	6.9.7	6.9.8	6.9.9	7.1.1	7.1.2	7.1.3	7.1.4	7.1.5	7.1.6
	7.1.7	7.1.8	7.1.9	7.2.1	7.2.2	7.2.3	7.2.4	7.2.5	7.2.6	7.2.7	7.2.8	7.2.9
40	7.3.1	7.3.2	7.3.3	7.3.4	7.3.5	7.3.6	7.3.7	7.3.8	7.3.9	7.4.1	7.4.2	7.4.3
	7.4.4	7.4.5	7.4.6	7.4.7	7.4.8	7.4.9	7.5.1	7.5.2	7.5.3	7.5.4	7.5.5	7.5.6
	7.5.7	7.5.8	7.5.9	7.6.1	7.6.2	7.6.3	7.6.4	7.6.5	7.6.6	7.6.7	7.6.8	7.6.9
	7.7.1	7.7.2	7.7.3	7.7.4	7.7.5	7.7.6	7.7.7	7.7.8	7.7.9	7.8.1	7.8.2	7.8.3

[illegible]

7.8.4	7.8.5	7.8.6	7.8.7	7.8.8	7.8.9	7.9.1	7.9.2	7.9.3	7.9.4	7.9.5	7.9.6
7.9.7	7.9.8	7.9.9	8.1.1	8.1.2	8.1.3	8.1.4	8.1.5	8.1.6	8.1.7	8.1.8	8.1.9
8.2.1	8.2.2	8.2.3	8.2.4	8.2.5	8.2.6	8.2.7	8.2.8	8.2.9	8.3.1	8.3.2	8.3.3
8.3.4	8.3.5	8.3.6	8.3.7	8.3.8	8.3.9	8.4.1	8.4.2	8.4.3	8.4.4	8.4.5	8.4.6
8.4.7	8.4.8	8.4.9	8.5.1	8.5.2	8.5.3	8.5.4	8.5.5	8.5.6	8.5.7	8.5.8	8.5.9
8.6.1	8.6.2	8.6.3	8.6.4	8.6.5	8.6.6	8.6.7	8.6.8	8.6.9	8.7.1	8.7.2	8.7.3
8.7.4	8.7.5	8.7.6	8.7.7	8.7.8	8.7.9	8.8.1	8.8.2	8.8.3	8.8.4	8.8.5	8.8.6
8.8.7	8.8.8	8.8.9	8.9.1	8.9.2	8.9.3	8.9.4	8.9.5	8.9.6	8.9.7	8.9.8	8.9.9
9.1.1	9.1.2	9.1.3	9.1.4	9.1.5	9.1.6	9.1.7	9.1.8	9.1.9	9.2.1	9.2.2	9.2.3
9.2.4	9.2.5	9.2.6	9.2.7	9.2.8	9.2.9	9.3.1	9.3.2	9.3.3	9.3.4	9.3.5	9.3.6
9.3.7	9.3.8	9.3.9	9.4.1	9.4.2	9.4.3	9.4.4	9.4.5	9.4.6	9.4.7	9.4.8	9.4.9
9.5.1	9.5.2	9.5.3	9.5.4	9.5.5	9.5.6	9.5.7	9.5.8	9.5.9	9.6.1	9.6.2	9.6.3
9.6.4	9.6.5	9.6.6	9.6.7	9.6.8	9.6.9	9.7.1	9.7.2	9.7.3	9.7.4	9.7.5	9.7.6
9.7.7	9.7.8	9.7.9	9.8.1	9.8.2	9.8.3	9.8.4	9.8.5	9.8.6	9.8.7	9.8.8	9.8.9
9.9.1	9.9.2	9.9.3	9.9.4	9.9.5	9.9.6	9.9.7	9.9.8	9.9.9			

JESSICA WOLFF\METABASIS 2450502 APPLICATION.DOC(METABASIS 2450502 APPLICATION.DOC)

SYNTHESIS OF COMPOUNDS OF FORMULA I

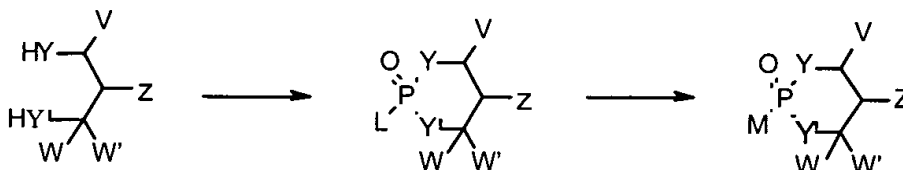
Synthesis of the compounds encompassed by the present invention includes: I). synthesis of prodrugs; and II). synthesis of substituted 1,3-hydroxyamines and 1,3-diamines.

5

I) SYNTHESIS OF PRODRUGS:

The following procedures on the preparation of prodrugs illustrate the general procedures used to prepare the prodrugs of the invention which apply to all phosphate, phosphonate- and phosphoramidate containing drugs. Prodrugs can be introduced at different stages of synthesis of a drug. Most often they are made at a later stage, because of the general sensitivity of these groups to various reaction conditions. Optically pure prodrugs containing single isomer at phosphorus center can be made either by separation of the diastereomers by a combination of column chromatography and/or crystallization, or by enantioselective synthesis of chiral activated phosphoramidate intermediates.

The preparation of prodrugs is further organized into 1) synthesis via activated P(V) intermediates; 2) synthesis via activated P(III) intermediates, 3) synthesis via phosph(on)ate diacid, and 4) miscellaneous methods. Some of the general synthetic methods described in here are utilized for phosphate and phosphonate ester synthesis. However, these methods are equally applicable in phosphoramidate case also when the nitrogen in the prodrug moiety is appropriately protected and/or substituted.

I.1 Synthesis via activated P(V) intermediate:I.1.a. Synthesis of activated P(V) intermediates:

Most preferred synthesis of phosphoramidate nucleotide prodrugs is the reaction of MH hydroxy group with 4-nitrophenyl phosphoramidate (where L is nitrophenyl) in presence of a strong base such as sodium hydride or lithium hydride. The activated precursor is prepared by reaction of commercially available 4-nitrophenyl phosphorochloridate with a substituted 1,3-aminoalcohol or 1,3-diamine. In general,

30

synthesis of phosphoroamidate prodrug is achieved by coupling the alcohol with the corresponding activated phosphoroamidate precursor for example,

Chlorophosphoroamidate (L=chloro) addition on to 5'-hydroxy of nucleoside is a well known method for preparation of phosphoroamidates (Stepanov *et al.*, *Tetrahedron Lett.*, 1989, 30, 5125). The activated precursor can be prepared by several well known methods. Chlorophosphoroamidates useful for synthesis of the prodrugs are prepared from the substituted-1,3-propanediamine or 1,3-aminoalcohol (Kirsten, *et al.*, *J. Org. Chem.*, 1997, 62, 6882). Chlorophosphoroamidate may also be made by oxidation of the corresponding P(III) intermediates (Ozaki, *et al.*, *Bull. Chem Soc. Jpn.*, 1989, 62, 3869) which are obtained by reaction of appropriately protected and/or substituted 1,3-aminoalcohol or 1,3-diamine with phosphorus trichloride. Alternatively, the chlorophosphoroamidate agent is made by treating substituted-1,3-aminoalcohol or 1,3-diamine with phosphorusoxychloride (Welch, *et al.*, *J. Org. Chem.*, 1990, 55, 5991).

Chlorophosphoroamidate species may also be generated in situ from corresponding phosphites (Jung, *et al.*, *Nucleosides and Nucleotides*, 1994, 13, 1597).

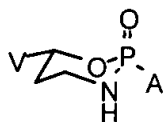
Phosphorofluoridate intermediate prepared from phosphoroamidic acid may also act as a precursor in preparation of cyclic prodrugs (Watanabe *et al.*, *Tetrahedron Lett.*, 1988, 29, 5763).

Phosphoramidates (L=NRR') can also be used as intermediates in the case of phosphoramidate prodrugs as in the synthesis of phosphate esters where the nitrogen is substituted or in the protected form. Monoalkyl or dialkylphosphoramidate (Watanabe, *et al.*, *Chem Pharm Bull.*, 1990, 38, 562), triazolophosphoramidate (Yamakage, *et al.*, *Tetrahedron*, 1989, 45, 5459) and pyrrolidinophosphoramidate (Nakayama, *et al.*, *J. Am. Chem. Soc.*, 1990, 112, 6936) are some of the known intermediates used for the preparation of phosphate esters. Another effective phosphorylating procedure is a metal catalyzed addition of cyclic chlorophosphoroamidate adduct of 2-oxazolone. This type of intermediate attains high selectivity in phosphorylation of primary hydroxy group in presence of secondary hydroxyl group (Nagamatsu, *et al.*, *Tetrahedron Lett.*, 1987, 28, 2375). These agents are obtained by reaction of a chlorophosphoroamidate with the amine or alternatively by formation of the corresponding phosphoramidite followed by oxidation.

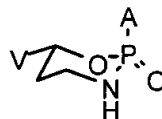
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I.1.b. Synthesis of chiral activated Phosphoramidate:

Phosphorylation of an enantiomerically pure substituted 1,3-aminoalcohol or 1,3-diamine with for example, a commercially available phosphorodichloridate R-OP(O)Cl₂, where RO is a leaving group, preferably aryl substituted with electron withdrawing groups, such as a nitro or a chloro, produces two diastereomeric intermediates that can be separated by a combination of column chromatography and/or crystallization. Such a method may also be utilized in preparing chiral chlorophosphoroamidate. Chiral phosphoramidate intermediates can be obtained by utilization of optically pure amine as the chiral auxiliary. This type of intermediate are known to undergo stereospecific substitution in the phosphate formation (Nakayama, *et al. J. Am. Chem. Soc.*, **1990**, 112, 6936). The relative configuration of the phosphorus atom can be determined by comparison of the ³¹P NMR spectra. The chemical shift of the equatorial phosphoryloxy moiety (trans-isomer) is always more upfield than the one of the axial isomer (cis-isomer) (Verkade, *et al, J. Org. Chem.*, **1977**, 42, 1549).



cis



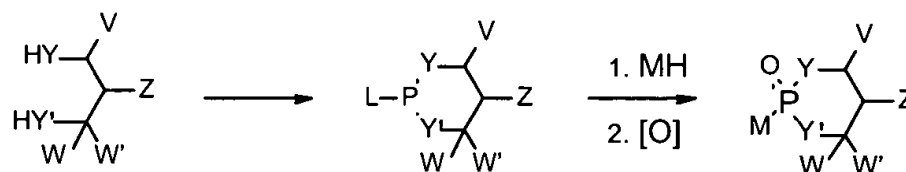
trans

I.1.c. Synthesis of Prodrugs Using Activated Phosphoramidates:

Coupling of activated phosphoramidates with alcohols (MH) is accomplished in the presence of an organic base. For example, Chloro phosphoramidates synthesized as described in the earlier section react with an alcohol in the presence of a base such as pyridines or N-methylimidazole. In some cases phosphorylation is enhanced by in situ generation of iodophosphoramidates from chloro (Stomberg, *et al., Nucleosides & Nucleotides.*, **1987**, 5: 815). Phosphoroflouridate intermediates have also been used in phosphorylation reactions in the presence of a base such as CsF or n-BuLi to generate cyclic prodrugs (Watanabe *et al., Tetrahedron lett.*, **1988**, 29, 5763). Phosphoramidate intermediates are shown to couple by transition metal catalysis (Nagamatsu, *et al., Tetrahedron Lett.*, **1987**, 28, 2375).

Reaction of the optically pure diastereomer of phosphoramidate intermediate with the hydroxyl of drug in the presence of an acid produces the optically pure phosphate prodrug by direct $S_N2(P)$ reaction (Nakayama, *et al.*, *J. Am. Chem. Soc.*, **1990**, 112, 6936). Alternatively, reaction of the optically pure phosphoramidate precursor with a fluoride source, preferably cesium fluoride or tetrabutylammonium fluoride, produces the more reactive phosphorofluoridate which reacts with the hydroxyl of the drug to give the optically pure prodrug configuration at the phosphorus atom (Ogilvie, *et al.*, *J. Am. Chem. Soc.*, **1977**, 99, 1277).

10 I.2 Synthesis via phosphoramidite intermediate:



I.2.a. Synthesis of activated P(III) intermediates:

Phosphorylation of hydroxy and amino groups is achieved using cyclic phosphorylating agents where the agent is at the P(III) oxidation state. One preferred phosphorylating agent is a chloro phosphoramidite (L'chloro). Cyclic chlorophosphoramidites are prepared under mild conditions by reaction of phosphorus trichloride with substituted 1,3-aminoalcohols or 1,3-diamines (Wada, *et al.*, *Tetrahedron Lett.*, **1990**, 31, 6363). Alternatively phosphoramidites can be used as the phosphorylating agent (Beaucage, *et al.*, *Tetrahedron*, **1993**, 49, 6123). Appropriately substituted phosphoramidites can be prepared by reacting cyclic chlorophosphoramidite with N,N-dialkylamine (Perich, *et al.*, *Aust. J. Chem.*, **1990**, 43, 1623. Perich, *et al.*, *Synthesis*, **1988**, 2, 142) or by reaction of commercially available dialkylaminophosphorochloridate with substituted propyl-1,3-aminoalcohols or 1,3-diamines.

I.2.b. Synthesis of chiral activated P(III) intermediate:

In the cases where unsymmetrical 1,3-aminoalcohols or 1,3-diamines are used, the cyclic phosphoramidites are expected to form a mixture of chiral isomers. When an optically active pure amino alcohol or diamine is used a chromatographically separable mixture of two stable diastereomers with the leaving group (NRR') axial and equatorial on

the phosphorous atom is expected (Brown *et al.*, *Tet.*, **1990**, 46, 4877). Pure diastereomers can usually be obtained by chromatographic separation. These intermediates may also be prepared through chirality induction by an optically active amine.

5 I.2.c. Synthesis of prodrugs Using Activated phosphoramidites:

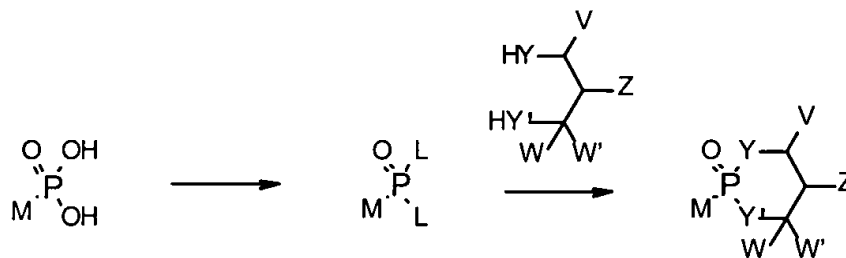
Appropriately substituted chlorophosphoramidites are used to phosphorylate alcohols on nucleosides in the presence of an organic base (e.g., triethylamine, pyridine).

Alternatively, the phosphoramidite can be obtained by coupling the nucleoside with a phosphoramidate in the presence of a coupling promoter such as tetrazole or

- 10 benzimidazolium triflate (Hayakawa *et al.*, *J. Org. Chem.*, **1996**, 61, 7996). Since condensation of alcohols with chloro phosphoramidites or phosphoramidites is an S_N2(P) reaction, the product is expected to have an inverted configuration. This allows for the stereoselective synthesis of cyclic phosphoramidites of prodrugs. Isomeric mixtures of phosphorylation reactions can also be equilibrated (e.g. thermal equilibration) to a more
- 15 thermodynamically stable isomer.

- The resulting phosphoramidites of prodrugs are subsequently oxidized to the corresponding phosphoramidate prodrugs using an oxidant such as molecular oxygen or t-butylhydroperoxide (Ozaki *et al.*, *Tetrahedron. Lett.*, **1989**, 30, 5899). Oxidation of optically pure P(III) intermediate is expected to stereoselectively provide optically active
- 20 prodrugs (Mikolajczyk, *et al.*, *J. Org. Chem.*, **1978**, 43, 2132. Cullis, P. M. *J. Chem. Soc., Chem Commun.*, **1984**, 1510, Verfurth, *et al.*, *Chem. Ber.*, **1991**, 129, 1627).

I.3 Synthesis of Phosphoramidate Prodrugs via Diacids:



- 25 Prodrugs of formula I are synthesized by reaction of the corresponding phosphodichloridate and an 1,3-aminoalcohol or 1,3-diamine (Khamnei, *et al.*, *J. Med. Chem.*, **1996**, 39:4109). For example, the reaction of a phosphodichloridate with substituted 1,3-aminoalcohols or diamines in the presence of base (such as pyridine,

Such reactive dichloridate intermediates, can be prepared from the corresponding acids and the chlorinating agents e.g. thionyl chloride (Starrett, *et al.*, *J. Med. Chem.*, **1994**, 1857), oxalyl chloride (Stowell, *et al.*, *Tetrahedron Lett.*, **1990**, 31: 3261), and phosphorus pentachloride (Quast, *et al.*, *Synthesis*, **1974**, 490). Alternatively, these dichlorophosphonates can also be generated from disilyl esters (Bhongle, *et al.*, *Synth. Commun.*, **1987**, 17: 1071) and dialkyl esters (Still, *et al.*, *Tetrahedron Lett.*, **1983**, 24: 4405; Patois, *et al.*, *Bull. Soc. Chim. Fr.*, **1993**, 130: 485).

Chiral phosphonoamidate prodrugs can be synthesized by either resolution (Pogatnic, *et al.*, *Tetrahedron Lett.*, **1997**, 38, 3495) or by chirality induction (Taapken, *et al.*, *Tetrahedron Lett.*, **1995**, 36, 6659; *J. Org. Chem.*, **1998**, 63, 8284).

Phosphorylation of an alcohol is also achieved under Mitsunobu reaction conditions using the cyclic 1',3'-propanyl phosphoramidic acid in the presence of triphenylphosphine and diethylazodicarboxylate (Kimura *et al.*, *Bull. Chem. Soc. Jpn.*, **1979**, 52, 1191). Furthermore, phosphate prodrugs can be made by conversion of nucleoside to the dichloridate intermediate with phosphoryl chloride in presence of triethylphosphite and quenching with substituted-1,3- aminoalcohols (Farquhar *et al.*, *J. Org. Chem.*, **1983**, 26, 1153).

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methylimidazole (Takaku, *et al.*, *J. Org. Chem.*, **1982**, 47, 4937). In addition, starting from a chiral cyclic phosphoramidic acid, obtained by chiral resolution (Wynberg, *et al.*, *J. Org. Chem.*, **1985**, 50, 4508) is also a source for chiral phosphoramidate prodrugs.

5 III. Synthesis of substituted 1,3-hydroxyamines and 1,3-diamines:

A large number of synthetic methods are available for the preparation of substituted 1,3-hydroxyamines and 1,3-diamines due to the ubiquitous nature of these functionalities in naturally occurring compounds. Following are some of these methods organized into: 1. synthesis of substituted 1,3-hydroxy amines; 2. synthesis of substituted
10 1,3-diamines and 3. synthesis of chiral substituted 1,3-hydroxyamines and 1,3-diamines.

III.1. synthesis of substituted 1,3-hydroxy amines:

A general synthetic procedure for 3-aryl-3-hydroxy-propan-1-amine type of prodrug moiety involves aldol type condensation of aryl esters with alkyl nitriles followed
15 by reduction of resulting substituted benzoylacetonitrile (Shih *et al.*, *Heterocycles*, **1986**, 24, 1599). The procedure can also be adapted for formation 2-substitutedaminopropanols by using substituted alkyl nitrile. In another approach, 3-aryl-3-amino-propan-1-ol type of prodrug groups are synthesized from aryl aldehydes by condensation of malonic acid in presence of ammonium acetate followed by reduction of resulting substituted α -amino
20 acids. Both these methods enable to introduce wide variety of substitution of aryl group (Shih, *et al.*, *Heterocycles*, **1978**, 9, 1277). In an alternate approach, α -substituted organolithium compounds of 1-amino-1-aryl ethyl dianion generated from styrene type of compounds undergo addition with carbonyl compounds to give variety of W, W' substitution by variation of the carbonyl compounds (Barluenga, *et al.*, *J. Org. Chem.*,
25 **1979**, 44, 4798).

Large number of synthetic methods are known for the preparation of racemic or chiral 1,3-diols. These methods may be utilized in the synthesis of corresponding substituted 1,3-aminoalcohols or 1,3 diamines by converting hydroxy functionality to a leaving group and treating with anhydrous ammonia or required primary or secondary amines (Corey, *et al.*, *Tetrahedron Lett.*, **1989**, 30, 5207; Gao, *et al.*, *J. Org. Chem.*, **1988**, 53, 4081). A similar transformation may also be achieved directly from alcohols in Mitsunobu type of reaction conditions (Hughes, D. L., *Org. React.*, **1992**, 42).

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A variety of synthetic methods are known to prepare the following types of 1,3-diols: a) 1-substituted; b) 2-substituted; and c) 1,2- or 1,3-annulated in their racemic or chiral form. Substitution of V, W, Z groups of formula I, can be introduced or modified either during synthesis of aminoalcohols or after the synthesis of prodrugs.

5

III.1a) 1-Substituted 1,3-Diols.

1,3-Dihydroxy compounds can be synthesized by several well known methods in literature. Aryl Grignard additions to 1-hydroxy propan-3-al give 1-aryl-substituted propan-1,3-diols. This method will enable conversion of various substituted aryl halides to 1-arylsubstituted-1,3-propane diols (Coppi, *et al.*, *J. Org. Chem.*, **1988**, 53, 911). Aryl halides can also be used to synthesize 1-substituted propanediols by Heck coupling of 1,3-diox-4-ene followed by reduction and hydrolysis (Sakamoto, *et al.*, *Tetrahedron Lett.*, **1992**, 33, 6845). Substituted 1,3-diols can be generated enantioselective reduction of vinyl ketone and hydoboration or by kinetic resolution of allylic alcohol. Variety of aromatic aldehydes can be converted to 1-substituted-1,3-diols by vinyl Grignard addition followed by hydroboration. Substituted aromatic aldehydes are also utilized by lithium-t-butylacetate addition followed by ester reduction (Turner., *J. Org. Chem.*, 1990, 55 4744). In another method, commercially available cinnamyl alcohols can be converted to epoxy alcohols under catalytic asymmetric epoxidation conditions. These epoxy alcohols are reduced by Red-Al to result in enantiomerically pure 1,3-diols (Gao, *et al.*, *J. Org. Chem.*, **1980**, 53, 4081). Alternatively, enantiomerically pure 1,3-diols can be obtained by chiral borane reduction of hydroxyethyl aryl ketone derivatives (Ramachandran, *et al.*, *Tetrahedron Lett.*, **1997**, 38 761). Pyridyl, quinoline, isoquinoline propan-3-ol derivatives can be oxygenated to 1-substituted-1,3-diol by N-oxide formation followed by rearrangement in acetic anhydride conditions (Yamamoto, *et al.*, *Tetrahedron*, **1981**, 37, 1871). Aldol condensation is another well described method for synthesis of the 1,3-oxygenated functionality (Mukaiyama, *Org. React.*, **1982**, 28, 203). Chiral substituted diols can also be made by enantioselective reduction of carbonyl compounds, by chiral aldol condensation or by enzyme promoted kinetic resolution.

30

III.1b) 2-Substituted 1,3-Diols:

Various 2-substituted-1,3-diols can be made from commercially available 2-(hydroxymethyl)-1,3-propane diol. Pentaerythritol can be converted to triol via

decarboxylation of diacid followed by reduction (Werle, *et al.*, *Liebigs. Ann. Chem.*, **1986**, 944) or diol-monocarboxylic acid derivatives can also be obtained by decarboxylation under known conditions (Iwata, *et al.*, *Tetrahedron Lett.* **1987**, 28, 3131). Nitrotriol is also known to give triol by reductive elimination (Latour, *et al.*, *Synthesis*, **1987**, 8, 742). The triol can be derivatised by mono acetylation or carbonate formation by treatment with alkanoyl chloride, or alkylchloroformate (Greene and Wuts, *Protective groups in organic synthesis*, John Wiley, New York, 1990). Aryl substitution can be affected by oxidation to aldehyde and aryl Grignard additions. Aldehydes can also be converted to substituted amines by reductive amination reaction.

III.1c) Cyclic-1,3- diols:

Compounds of formula 1 where V - Z or V - W are fused by four carbons are made from Cyclohexane derivatives. Commercially available *cis*, *cis*-1,3,5-cyclohexane triol can be used as is or modified as described in case of 2-substituted propan-1,3-diols to give various analogues. These modifications can either be made before or after ester formation. Various 1,3-cyclohexane diols can be made by Diels-Alder methodology using pyrone as diene (Posner, *et al.*, *Tetrahedron Lett.*, **1991**, 32, 5295). Cyclohexyl diol derivatives are also made by nitrile oxide-olefin additions (Curran, *et al.*, *J. Am. Chem. Soc.*, **1985**, 107, 6023). Alternatively, cyclohexyl precursors are also made from commercially available quinic acid (Rao, *et al.*, *Tetrahedron Lett.*, **1991**, 32, 547.)

III.2. synthesis of substituted 1,3-diamines:

Substituted 1,3-diamines are synthesized starting from variety of substrates. Arylglutaronitriles can be transformed to 1-substituted diamines by hydrolysis to amide and Hoffman rearrangement conditions (Bertochio, *et al.*, *Bull. Soc. Chim. Fr.*, **1962**, 1809). Whereas, malononitrile substitution will enable variety of Z substitution by electrophile introduction followed by hydride reduction to corresponding diamines. In another approach, cinnamaldehydes react with hydrazines or substituted hydrazines to give corresponding pyrazolines which upon catalytic hydrogenation result in substituted 1,3-diamines (Weinhardt, *et al.*, *J. Med. Chem.*, **1985**, 28, 694). High trans-diastereoselectivity of 1,3-substitution is also attainable by aryl Grignard addition on to pyrazolines followed by reduction (Alexakis, *et al.*, *J. Org. Chem.*, **1992**, 576, 4563). 1-Aryl-1,3-diaminopropanes are also prepared by diborane reduction of 3-amino-3-

arylacrylonitriles which in turn are made from nitrile substituted aromatic compounds (Dornow, *et al.*, *Chem Ber.*, **1949**, 82, 254). Reduction of 1,3-diimines obtained from corresponding 1,3-carbonyl compounds are another source of 1,3-diamine prodrug moiety which allows a wide variety of activating groups V and/or Z (Barluenga, *et al.*, *J. Org. Chem.*, **1983**, 48, 2255).

III.3. Synthesis of chiral substituted 1,3-hydroxyamines and 1,3-diamines:

Enantiomerically pure 3-aryl-3-hydroxypropan-1-amines are synthesized by CBS enantioselective catalytic reaction of β -chloropropiophenone followed by displacement of halo group to make secondary or primary amines as required (Corey, *et al.*, *Tetrahedron Lett.*, **1989**, 30, 5207). Chiral 3-aryl-3-amino propan-1-ol type of prodrug moiety may be obtained by 1,3-dipolar addition of chirally pure olefin and substituted nitron of arylaldehyde followed by reduction of resulting isoxazolidine (Koizumi, *et al.*, *J. Org. Chem.*, **1982**, 47, 4005). Chiral induction in 1,3-polar additions to form substituted isoxazolidines is also attained by chiral phosphine palladium complexes resulting in enantioselective formation of δ -amino alcohol (Hori, *et al.*, *J. Org. Chem.*, **1999**, 64, 5017). Alternatively, optically pure 1-aryl substituted amino alcohols are obtained by selective ring opening of corresponding chiral epoxy alcohols with desired amines (Canas *et al.*, *Tetrahedron Lett.*, **1991**, 32, 6931).

Several methods are known for diastereoselective synthesis of 1,3-disubstituted aminoalcohols. For example, treatment of (E)-N-cinnamyltrichloroacetamide with hypochlorous acid results in *trans*-dihydrooxazine which is readily hydrolysed to *erythro*- β -chloro- α -hydroxy- δ -phenylpropanamine in high diastereoselectivity (Commercon *et al.*, *Tetrahedron Lett.*, **1990**, 31, 3871). Diastereoselective formation of 1,3-aminoalcohols is also achieved by reductive amination of optically pure 3-hydroxy ketones (Haddad *et al.*, *Tetrahedron Lett.*, **1997**, 38, 5981). In an alternate approach, 3-aminoketones are transformed to 1,3-disubstituted aminoalcohols in high stereoselectivity by a selective hydride reduction (Barluenga *et al.*, *J. Org. Chem.*, **1992**, 57, 1219).

All the above mentioned methods can also be applied to prepare corresponding V-Z or V-W annulated chiral aminoalcohols. Furthermore, such optically pure amino alcohols are also a source to obtain optically pure diamines by the procedures described earlier in the section.

Formulations

Compounds of the invention are administered orally in a total daily dose of about 0.1 mg/kg/dose to about 100 mg/kg/dose, preferably from about 0.3 mg/kg/dose to about 30 mg/kg/dose. The most preferred dose range is from 0.5 to 10 mg/kg (approximately 1 to 20 nmoles/kg/dose). The use of time-release preparations to control the rate of release of the active ingredient may be preferred. The dose may be administered in as many divided doses as is convenient. When other methods are used (e.g. intravenous administration), compounds are administered to the affected tissue at a rate from 0.3 to 300 nmol/kg/min, preferably from 3 to 100 nmoles/kg/min. Such rates are easily maintained when these compounds are intravenously administered as discussed below.

For the purposes of this invention, the compounds may be administered by a variety of means including orally, parenterally, by inhalation spray, topically, or rectally in formulations containing pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used here includes subcutaneous, intravenous, intramuscular, and intraarterial injections with a variety of infusion techniques. Intraarterial and intravenous injection as used herein includes administration through catheters. Oral administration is generally preferred.

Pharmaceutical compositions containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

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The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain 20 to 2000 μmol (approximately 10 to 1000 mg) of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions. It is preferred that the pharmaceutical composition be prepared which provides easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion should contain from about 0.05 to about 50 μmol (approximately 0.025 to 25 mg) of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a

rate of about 30 mL/hr can occur.

As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropyl methylcellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach. This is particularly advantageous with the compounds of formula I when such compounds are susceptible to acid hydrolysis.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain antioxidants, buffers,

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bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, or an appropriate fraction thereof, of a drug.

It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors including the activity of the specific compound employed; the age, body weight, general health, sex and diet of the individual being treated; the time and route of administration; the rate of excretion; other drugs which have previously been administered; and the severity of the particular disease undergoing therapy, as is well understood by those skilled in the art.

EXAMPLES

The prodrug compounds of this invention, their intermediates, and their preparation can be understood further by the examples which illustrate some of the processes by which these compounds are prepared. These examples should not however be construed as specifically limiting the invention and variations of the compounds, now known or later developed, are considered to fall within the scope of the present invention as hereinafter claimed.

Compounds of formula I are prepared using procedures detailed in the following examples.

Example 1:

General procedure for phosphoramidate prodrugs of phosphonic acid containing drugs:

A suspension of 5-Isobutyl-2-methyl-4-(2-(5-phosphono)furan-2-ylthiazole (200mg, 0.6 mmol) in 2.5 mL of thionyl chloride was heated at reflux temperature for 4 h. The reaction mixture was cooled and evaporated to dryness followed by azeotropic with toluene. To the solution of the resulting residue in 4 ml of methylene chloride was added a

After stirring at 25 °C for 4 h the reaction was evaporated and coevaporated with toluene. The crude product was chromatographed by eluting with 10% methanol-dichloromethane to give 52 mg (20.8%) of a less polar isomer and 48 mg (19.2%) of a more polar isomer.

10 **1.1:** 5-Isobutyl-2-methyl-4-{2-[5-(1-phenyl-1,3-propyl)phosphorimido]furanlyl}thiazole, less polar isomer. $R_f=0.76$ in 10% MeOH/CH₂Cl₂. Anal. Calcd. for C₂₁H₂₅N₂O₃PS+0.25H₂O+0.1HCl: C: 59.40; H: 6.08; N: 6.60. Found: C: 59.42; H: 5.72; N: 6.44.

1.2: 5-Isobutyl-2-methyl-4-{2-[5-(1-phenyl-1,3-propyl)phosphorimido]furanlyl}thiazole, more polar isomer. $R_f=0.7$ in MeOH/CH₂Cl₂. Anal. Calcd. for C₂₁H₂₅N₂O₃PS+0.25H₂O: C: 59.91; H: 6.1; N: 6.65. Found: C: 60.17; H: 5.81, N: 6.52.

15 **1.3:** 2-Amino-5-isobutyl-4-{2-[5-(1-phenyl-1,3-propyl)phosphoramido]furanlyl}thiazole. $R_f = 0.53$ (silica, 9:1 CH₂Cl₂/MeOH). Anal. calcd. for C₂₀ H₂₄ N₃ O₃ P S + 0.2CH₂Cl₂ + 0.25H₂O : C: 55.27; H: 5.72; N: 9.57. Found: C: 55.03; H: 5.42; N: 9.37.

20 **1.4:** 2-Amino-5-isobutyl-4-{2-[5-(1-phenyl-1,3-propyl)phosphoramido]furanlyl}thiazole, less polar isomer. $R_f = 0.57$ (silica, 9:1 CH₂Cl₂/MeOH). Anal. calcd. for C₂₀ H₂₄ N₃ O₃ P S + 0.15 CH₂Cl₂: C: 56.26; H: 5.69; N: 9.77. Found: C: 56.36; H: 5.46; N: 9.59.

1.5: 2-Amino-5-methylthio-4-{2-[5-(1-phenyl-1,3-propyl)phosphoramido]furanlyl}thiazole, less polar isomer. $R_f = 0.59$ (silica, 9:1 CH₂Cl₂/MeOH). Anal. calcd. for C₁₇ H₁₈ N₃ O₃ P S₂ + 0.4HCl: C: 48.38; H: 4.39; N: 9.96. Found: C: 48.47; H: 4.21; N: 9.96.

25 **1.6:** 2-Amino-5-methylthio-4-{2-[5-(1-phenyl-1,3-propyl)phosphoramido]furanlyl}thiazole, more polar isomer. $R_f = 0.56$ (silica, 9:1 CH₂Cl₂/MeOH). Anal. calcd. for C₁₇ H₁₈ N₃ O₃ P S₂: C: 50.11; H:4.45; N:10.31. Found: C: 49.84; H: 4.19; N: 10.13.

30 **1.7:** 2-Amino-5-methylthio-4-{2-[5-(N-methyl-1-phenyl-

1,3-propyl)phosphoramido]furanyl}thiazole. Rf = 0.56 (silica, 9:1 CH₂Cl₂/MeOH). Anal. cald. for C₁₈ H₂₀ N₃ O₃ P S₂ + .25HCl

C: 50.21; H: 4.74; N: 9.76. Found: C: 50.31; H: 4.46; N: 9.79.

1.8: 2-Amino-5-isobutyl-4-{2-[5-(1-phenyl-1,3-

5 propyl)-N-acetylphosphoramido]furanyl}thiazole. Rf=0.62 (silica, 9:1 CH₂Cl₂/MeOH). Anal. cald. for C₂₂ H₂₆ N₃ O₄ P S + 1.25H₂O: C: 54.82; H: 5.96; N: 8.72. Found: C: 55.09; H: 5.99; N: 8.39.

1.9: 2-Amino-5-isobutyl-4-{2-[5-(1-[2,4-dichlorophenyl]-

propan-1,3-yl)phosphoramido]furanyl}thiazole. mp 235-237 C (decomp). Rf=0.35 (silica, 10 3:2 EtOAc/CH₂Cl₂). Anal. cald. for C₂₀ H₂₂ Cl₂ N₃ O₃ P S: C: 49.39 H: 4.56; N: 8.64. Found: C: 49.04; H: 4.51; N: 8.37.

1.10: 188 - 189 9-{2-[1-phenyl-1-aza-3-oxa-

2-phosphorinanyl-2-methyloxy]ethyl}adenine. mp 188 - 189 C. Rf = 0.25 (silica, 9:1 15 CHCl₂/MeOH). Anal. cald. for C₁₇ H₂₁ N₆ O₃ P + 0.5 H₂ O: C:51.38; H: 5.58; N: 21.15. Found: C: 51.05; H: 5.28; N: 20.77.

1.11: 2-Amino-5-isobutyl-4-{2-[5-(1-(3-chlorophenyl)-

propan-1,3-yl)phosphoradiamido]furanyl}thiazole. Light yellow solid; Rf = 0.45 (Silica, 5:95 Methanol/dichloromethane); ¹H NMR (200 MHz, DMSO-d₆) d 20 7.35 (m, 4H); 7.1 (m, 1H), 6.6 (m, 1H); 5.55 (m, 1H), 2.8 (d, 2H), 0.9 (d, 6H).

Example 2:

Step A:

A solution of 3-amino-3-phenyl-1-propanol (1 g, 6.6 mmol) and triethylamine (3 25 ml, 21.8 mmol) in tetrahydrofuran (60 ml) was added dropwise over 20 minutes into a solution of 4-nitrophenyl phosphorodichloridate in tetrahydrofuran at 0°C. A white precipitate quickly forms. The yellow heterogeneous mixture was stirred at 0°C for 1 hour then warmed slowly to rt. After stirring at rt for 60 hours, the precipitate was dissolved with water (40 ml). The clear yellow solution was concentrated to ca 40 ml. The resulting 30 mixture was diluted with a saturated aqueous solution of sodium bicarbonate and extracted twice with ethyl acetate. The combined organic extracts were washed with a saturated aqueous solution of sodium bicarbonate and brine. The aqueous washes were back extracted and the organic extracts combined. The combined organic extracts were dried

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over sodium sulfate, concentrated and purified by column chromatography (silica gel, hexanes/ethyl acetate) to give fast eluting trans isomer (995 mg, 45%) and slow eluting cis isomer (1 g, 45 %) and);

5 Step B:

A mixture of 2',3'-dideoxy-adenosine (50 mg, 0.21 mmol), cesium fluoride (323 mg, 2.1 mmol) and fast eluting (4-Nitrophenoxy)-4-phenyl-1,3,2-oxazaphosphorinan-2-one (142 mg, 0.4 mmol) in *tert*-butanol was heated at 80°C. After 48 hours, more (4-Nitrophenoxy)-4-phenyl-1,3,2-oxazaphosphorinan-2-one (70 mg) was added and stirring was continued at 80°C. After 5 days, the cooled yellow heterogeneous mixture was diluted with 50/50 methanol/dichloromethane, filtered through a pad of silica and rinsed with 50/50 methanol/dichloromethane. The combined filtrates were concentrated and the residue was purified by column chromatography (silica gel, methanol/dichloromethane) to give the prodrug which was re crystallized from dichloromethane and hexanes : 42 mg (47% yield).

The following compounds are prepared in this manner:

2.1 2',3'-Dideoxy-5'-*O*-(2-oxo-4-phenyl-1,3,2-oxazaphosphorin-2-yl)-adenosine. mp 119-122. R_f = 0.25 (silica gel, 1/9 methanol/dichloromethane). Anal. Cald. for C₁₉H₂₃N₆O₄P + 0.9 mol dichloromethane: C: 47.16; H: 4.93; N: 16.58. Found: C: 47.03; H: 4.98; N: 16.61.

2.2 3'-Azido-3'-deoxy-5'-*O*-(4-phenyl-2-oxo-1,3,2-oxazaphosphorin-2-yl)-thymidine. mp 85-105. R_f = 0.5 (silica gel, 1/9 methanol/dichloromethane). Anal. cald. for C₁₉H₂₃N₆O₆P: C: 49.35; H: 5.01; N: 18.17. Found: C: 49.32; H: 5.17; N: 17.89.

Example 3:

Step A:

Same as step A of example 2.

30 Step B:

Lithium hydride (15 mg, 1.52 mmol) was added to a solution of 9-β-D-arabinofuranosyl-adenine (100 mg, 0.37 mmol) and fast eluting (4-nitrophenoxy)-4-phenyl-1,3,2-oxazaphosphorinan-2-one (250 mg, 0.74 mmol) in dimethylformamide at

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5 18 mg (11 % yield);

3.1 5'-O-(2-oxo-4-Phenyl-1,3,2-oxazaphosphorin-2-yl)-9-β-D-arabinofuranosyl-adenine.

The following compounds are made in a similar manner with the exception that the reactions were neutralized with 1N solution of hydrochloric acid during work up.

3.2 9-[(2-(4-Phenyl-2-oxo-1,3,2-oxazaphosphorinan-2-oxo)-ethoxy)methyl]-guanine :

3.3 2'-Deoxy-5-fluoro-5'-*O*-(4-phenyl-2-oxo-1,3,2-oxazaphosphorin-2-yl)-uridine.

25 General procedure for formation of nucleotide prodrugs by P(III) intermediates:

To a solution of 1(aryl)- 1- (1-alkyl or acyl amino) propanol (1mmol) in dichloromethane (10mL) is added phosphorus trichloride at 0 °C. The reaction is warmed to room temperature and allowed to stir for 3h. Reaction mixture is concentrated, azeotroped with toluene (2X10mL) and dried. Crude chlorophosphoramidite is used in the next step without further purification.

To a solution of nucleoside (1mmol) in DMF (10 mL) is added diisopropylethylamine (2mmol) at -40 °C. To this mixture is added crude cyclic

5

General procedure for preparation of 3-Aryl-3-aminopropanols from aryl aldehydes:

Step A:

15 Step B:

20

25 General procedure for preparation of 3-Aryl-3-hydroxypropylamines from epoxy
cinnamyl alcohols:

Step A:

30

5 Step B:

10

15

20

Step A:

25

Step B:

A solution of 3-phenylpyrazoline (10 mmol) in 50 mL of AcOH and 10 mL of 10% HCl is hydrogenated at atmospheric pressure over 500mg of 5% Pt/C. The reaction is stirred under hydrogen for 6 h. The catalyst is removed by filtration and the filtrate is concentrated. The crude product is purified by column to give pure 3-Aryl-propylenediamines derivative.

Example 8:

General procedure for 1,3-amino alcohols for 2-substituted prodrugs (V, W and W'=H and

10 Z= -CHR²OH):

Step A:

To a solution of commercially available 2-(hydroxymethyl)-1,3-propane diol (1 mmol) in pyridine (5mL) is added methanesulfonyl chloride (1 mmol) and stirred at room temperature until the reaction is complete. The mixture is concentrated, extracted and product is purified by column chromatography.

Step B:

A solution of 2-(methanesulfonyloxymethylene)-1,3-propane diol (1mmol) and ammonium hydroxide (10mL, 30% in water) in THF (10mL) is heated at 100 °C in a sealed reactor. After cooling, the mixture is concentrated, extracted and product is purified by column chromatography to give 2-substituted-1,3-amino alcohol.

Appropriate amines may be used in step B to vary -NR⁶ substitution.

2-substituted-1,3-diamines may also be synthesized using Step A and B from 2-(hydroxymethyl)-1,3-propane diol using appropriate stoichiometry of reagents.

Step C:

25 Prodrugs of 2-substituted-1,3-amino alcohols or 2-substituted-1,3-diamines are synthesized by following coupling procedures as described in example 1 or example 2 (steps A and B) or example 3 (step B) or example 4 depending on the parent compound.

Example 9:

30 General procedure for cyclic 1,3-amino alcohols (together V and W are connected):

Step A:

Commercially available cis, cis-1,3,5-cyclohexane triol is converted to a mono mesylate as described in example 8, step A.

Step B:

cis, cis-1-(methylsulfonyloxy) cyclohexane-3,5-diol is transformed to corresponding amino alcohol as described in example 8, step B.

Appropriate amines may be used in step B to vary -NR⁶ substitution.

- 5 1-Hydroxy-3,5-diamino cyclohexane may also be synthesized using Step A and B from cis, cis-1,3,5-cyclohexane triol using appropriate stoichiometry of reagents.

Step C:

- Prodrugs of cyclohexyl-1,3-amino alcohols or 1,3-diamines are synthesized by following procedures as described in example 1 or example 2 (steps A and B) or example 10 3 (step B) or example 4 depending on the parent compound.

Example 10Preparation of 2-substituted 4-[2'-(5'-phosphono)furanyl]thiazoles.Step A.

- 15 A solution of furan (1.3 mmole) in toluene was treated with 4-methyl pentanoic acid (1 mmole), trifluoroacetic anhydride (1.2 mmole) and boron trifluoride etherate (0.1 mmole) at 56 °C for 3.5 h. The cooled reaction mixture was quenched with aqueous sodium bicarbonate (1.9 mmole), filtered through a celite pad. Extraction, evaporation and distillation gave 2-[(4-methyl-1-oxo)pentyl]furan as a brown oil (bp 65 - 77 °C, 0.1 20 mmHg).

Step B.

- A solution of 2-[(4-methyl-1-oxo)pentyl]furan (1 mmole) in benzene was treated with ethylene glycol (2.1 mmole) and p-toluenesulfonic acid (0.05 mmole) at reflux for 60 h while removing water via a Dean-Stark trap. Triethyl orthoformate (0.6 mmole) was 25 added and resulting mixture was heated at reflux for an additional hour. Extraction and evaporation gave 2-(2-furanyl)-2-[(3-methyl)butyl]-1,3-dioxolane as an orange liquid.

Step C.

- A solution of 2-(2-furanyl)-2-[(3-methyl)butyl]-1,3-dioxolane (1 mmole) in THF was treated with TMEDA (1 mmole) and nBuLi (1.1 mmole) at -45 °C, and the resulting 30 reaction mixture was stirred at -5 to 0 °C for 1 h. The resulting reaction mixture was cooled to -45 °C, and cannulated into a solution of diethyl chlorophosphate in THF at -45

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°C. The reaction mixture was gradually warmed to ambient temperature over 1.25 h. Extraction and evaporation gave 2-[2-(5-diethylphosphono)furanyl]-2-[(3-methyl)butyl]-1,3-dioxolane as a dark oil.

Step D.

5 A solution of 2-[2-(5-diethylphosphono)furanyl]-2-[(3-methyl)butyl]-1,3-dioxolane (1 mmole) in methanol was treated with 1 N hydrochloric acid (0.2 mmole) at 60 °C for 18 h. Extraction and distillation gave 5-diethylphosphono-2-[(4-methyl-1-oxo)pentyl]furan as a light orange oil (bp 152 - 156 °C, 0.1 mmHg).

Step E.

10 A solution of compound gave 5-diethylphosphono-2-[(4-methyl-1-oxo)pentyl]furan (1 mmole) in ethanol was treated with copper (II) bromide (2.2 mmole) at reflux for 3 h. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness. The resulting dark oil was purified by chromatography to give 5-diethylphosphono-2-[(2-bromo-4-methyl-1-oxo)pentyl]furan as an orange oil.

15 Step F. A solution of 5-diethylphosphono-2-[(2-bromo-4-methyl-1-oxo)pentyl]furan (1 mmole) and thiourea (2 mmole) in ethanol was heated at reflux for 2 h. The cooled reaction mixture was evaporated to dryness and the resulting yellow foam was suspended in saturated sodium bicarbonate and water (pH = 8). The resulting yellow solid was collected through filtration to give 2-amino-5-isobutyl-4-[2-(5-diethylphosphono)furanyl]thiazole.

Step G.

20 A solution of 2-amino-5-isobutyl-4-[2-(5-diethylphosphono)-furanyl]thiazole (1 mmole) in methylene chloride was treated with bromotrimethylsilane (10 mmole) at 25 °C for 8 h. The reaction mixture was evaporated to dryness and the residue was suspended in water. The resulting solid was collected through filtration to give 2-amino-5-isobutyl-4-[2-(5-phosphono)furanyl]thiazole as an off-white solid.

10.1 2-amino-5-isobutyl-4-[2'-(5'-phosphono)furanyl]thiazole mp > 250 °C. Anal. calcd. for C₁₁H₁₅N₂O₄PS + 1.25HBr: C: 32.75; H: 4.06; N: 6.94. Found: C: 32.39; H: 4.33; N: 7.18.

The following compounds were prepared according to similar procedure:

10.2 2-Methyl-5-isobutyl-4-[2'-(5'-phosphono)furanyl]thiazole. Anal. calcd. for $C_{12}H_{16}NO_4PS + HBr + 0.1CH_2Cl_2$: C: 37.20; H: 4.44; N: 3.58. Found: C: 37.24; H: 4.56; N: 3.30.

- 5 **10.3** 2-Amino-5-methylthio-4-[2'-(5'-phosphono)furanyl]thiazole. mp 181-184 °C. Anal. calcd. for $C_8H_9N_2O_4PS_2 + 0.4H_2O$: C: 32.08; H: 3.30; N: 9.35.

Found: C: 32.09; H: 3.31; N: 9.15.

Example 11:

- 10 General procedure for the synthesis of cyclophosphamide analogs:
(Boyd, *et al.*, *J. Med. Chem.* **1980**, 23, 372)

A solution of bis(2-chloroethyl)phosphoramidic dichloride (10 mmol) in ethyl acetate (100 mL) is added dropwise to a solution of commercial 3 methylamino-1-phenyl-1-propanol (10 mmol) and triethylamine (20 mmol) in ethyl acetate (100 mL) at rt. After
15 stirring at rt for 3 days, the salts are filtered off and the combined filtrates are concentrated under vacuum. The crude product is purified by column chromatography on silica.

Example 12:

Phosphoramidate Prodrugs of 1-Methylamino-1-phenyl-3-propanol

- 20 Step A:

The reagent N-methyl-2-(4-nitrophenoxy)-2-oxo-6-phenyl-1,3,2-oxazaphosphorinane was made using the same procedure as the one described for step A of example 2:

- 25 Fast eluting isomer: White solid, mp 135°C; $R_f = 0.4$ (Silica, Ethyl acetate/Hexanes 1/1). Slow eluting isomer: White solid, mp 128-129; $R_f = 0.15$ (Silica, Ethyl acetate/Hexanes 1/1).

Step B:

- 30 The coupling step with the nucleoside was accomplished in a manner similar to the one described in step B of example 3 except that the reaction mixture was stirred at 100°C for 5 hours.

The following compound was prepared in this manner:

12.1: 9-[(2-(N-Methyl-2-oxo-6-phenyl-1,3,2-oxazaphosphorin-2-oxy)-ethoxy)methyl]-
guanine: pale yellow amorphous solid; $R_f = 0.45$ (Silica, Methanol/dichloromethane 2/8);
 ^1H NMR (200 MHz, DMSO d_6) δ 7.8 (bs, 1 H); 7.3 (bs, 5 H), 6.75 (bs, 2 H, exchangeable
with D_2O); 5.35 (bs, 3 H).

5

The following compound was prepared in this manner:

12.2: 5-(N-Methyl-2-oxo-6-phenyl-1,3,2-oxazaphosphorin-2-yl)-9- β -D-
arabinofuranosyladenine: Off white amorphous solid; $R_f = 0.2$ (Silica,

10 Methanol/dichloromethane 1/9); ^1H NMR (200 MHz, DMSO d_6) δ 7.4-7.1 (m, 5 H); 5.75
(s, 1 H), 5.25 (m, 1 H); 4.8 (m, 1 H).

Example 13

Phosphoramidate prodrugs of 3-amino-1-hydroxy-1-phenyl propane

15 Step A:

A mixture of 3-phenyl-3-hydroxypropylmethanesulfonate (1.71 g, Gao, *et al.*, *J.*
Org. Chem., **1988**, 53, 4081), tetrahydrofuran (10 mL) and 28% ammonium hydroxide (30
mL) was heated in a bomb at 65°C for 16 hours. After cooling, the volatiles were
eliminated to leave the aqueous solution which was extracted with ether. The aqueous
20 layer was concentrated under reduced pressure, azeotroped twice with anhydrous
acetonitrile and dried under vacuum to give 3-amino-1-phenyl-1-propanol (1.04 g) as a
white solid: mp $92-94^\circ\text{C}$, $R_f = 0.1$ (silica, methanol/ethyl acetate 1/1).

Step B:

The reagent 2-(4-nitrophenoxy)-2-oxo-6-phenyl-1,3,2-oxazaphosphorinane (**13.1**)
25 was made using the same procedure as the one described for step A of example 2:

Fast eluting isomer: $R_f = 0.6$ (Silica, Ethyl acetate), ^1H NMR (200 MHz, DMSO d_6)
 δ 8.22 (m, 2 H); 7.48 (m, 2 H), 7.35 (m, 5 H); 5.52 (m, 1 H).

Step C:

The coupling step with the nucleoside is accomplished in a manner similar to the
30 one described in step B of example 3.

The following compounds are made in this manner:

9-[(2-(N-Methyl-2-oxo-6-phenyl-1,3,2-oxazaphosphorin-2-oxy)-ethoxy)methyl]-guanine;

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Examples of use of the method of the invention includes the following. It will be understood that these examples are exemplary and that the method of the invention is not limited solely to these examples.

BIOLOGICAL EXAMPLES

Methods: Aliquots of a 10 µg/mL solution of Compound **1.3** in potassium phosphate buffers at pH 3 and 7 (room temperature) were sampled hourly for 12 hours. Samples were analyzed by reverse phase HPLC with use of a Beckman Ultrasphere C8 column (4.6 X 250 mm). The column was equilibrated and eluted with a gradient from 50 mM sodium phosphate pH 5.5 to 80% acetonitrile at a flow rate of 1.0 mL/min. Detection was at 254 nm. Under these conditions, Compound **1.3** was separated from 2-Amino-5-isobutyl-4-[2- (5-phosphonomonoamide)furanyl]thiazole, and from 2-Amino-5-isobutyl-4-(2-furanyl)thiazole (retention times = 17, 15.8, and 15.6 min., respectively).

Results: The half-life of Compound **1.3** at pH 3.0 was 3.8 h; prodrug decomposed to an unidentified metabolite at this pH. Compound **1.3** was fully stable at pH 7.0; there was no evidence of decomposition throughout the 12-hour incubation.

Example B: Stability of Phosphoramidate Prodrugs to Esterases, Phosphatases, Adenosine Deaminase, and Plasma

Methods: Carboxylesterase (porcine liver) and alkaline phosphatase (calf intestinal mucose) are purchased from Sigma Chemical Co. (St. Louis, MO). Carboxylesterase activity is measured in 0.1 M Tris-HCl buffer at pH 8.0. Activity towards p-nitrophenyl acetate, a known substrate and positive control in the reactions is measured as described for example by Matsushima M., et al. [FEBS Lett. (1991)293(1-2): 37-41]. Alkaline phosphatase activity is measured in a 0.1 M diethanolamine buffer, pH

9.8, containing 0.5 mM MgCl₂. Activity towards p-nitrophenyl phosphate, a known substrate and positive control in the reactions, is measured as described [e.g. Brenna O., et al (1975) Biochem J. 151(2): 291-6]. Adenosine deaminase activity is measured as described by e.g. Gustin NC, et al. [Anal. Biochem. (1976) 71: 527-532]. Adenosine is used as a positive control in these reactions. Plasma is prepared by centrifugation from fresh, heparinized rat or human blood. Prodrugs are incubated at a concentration of, for example, 25 μM in appropriate reaction mixtures containing carboxylesterase, alkaline phosphatase, adenosine deaminase, or rat or human plasma. In the case of the esterase, phosphatase, and adenosine deaminase assays, parallel reactions are run with known substrates of the enzymes as described.

Aliquots are removed from the reaction mixture at various time points and the reaction stopped by addition of methanol to 60%. Following centrifugation and filtration, the methanolic aliquots are analyzed for generation of MPO₂-(NHR⁶) or other metabolites by standard reverse phase, ion-pairing, or ion exchange HPLC methods.

Results: MPO₂-(NHR⁶) or metabolites thereof are not detected following exposure of the prodrugs to carboxylesterase, alkaline phosphatase or plasma.

Example C: Activation of Phosphoramidate Prodrugs by Rat Liver Microsomes

Methods: The microsomal fraction is prepared from fresh, saline-perfused rat liver. Liver tissue is homogenized in three volumes (w/v) of 0.2 M KH₂PO₄ buffer pH 7.5, containing 2mM MgCl₂ and 1 mM EGTA. The homogenate is centrifuged at 10,000 g for 1 hour and the supernatant recovered. The supernatant fraction is then recentrifuged at 100,000 g to pellet the microsomal fraction. The pellet is resuspended in homogenization buffer and recentrifuged. This process is repeated twice to ensure complete removal of cytosolic enzyme activities. After the last centrifugation, the microsomal pellet is resuspended in homogenization buffer at a final protein concentration of about 14 mg/ml. Reaction mixtures (0.5 ml) consist of 0.2 M KH₂PO₄ pH 7.5, 13 Mm glucose-6-phosphate, 2.2 mM NADP⁺, 1 unit of glucose-6-phosphate dehydrogenase, 0-2.5 mg/ml microsomal protein and 100 μM 7.1. Reactions are carried out at 37 °C. Aliquots are removed from the reaction mixtures at appropriate time points, and extracted with 60% methanol. The methanolic extracts are centrifuged at 14,000 rpm, and filtered (0.2 μM) prior to analysis by HPLC. Standard HPLC techniques

including reverse phase, ion-pairing, or ion exchange chromatography are used. Eluted peaks are quantified relative to authentic standards of known concentration.

Alternatively, the activation of prodrugs is monitored by the depletion of NADPH, an essential cofactor in the reaction. This assay is performed in reactions mixtures consisting of 0.2 M KH_2PO_4 , 0.22 mM NADPH, 0-2.5 mg/ml microsomal protein, and 100 μM 28.4 or 30.1. Reactions are monitored spectrophotometrically at 340 nm. A decrease in absorbance is indicative of cofactor depletion and thus of the enzymatic oxidation of prodrug to $\text{MPO}_2\text{-(NHR}^{6-})$.

Results: Prodrugs are converted to $\text{MPO}_2\text{-(NHR}^{6-})$ in the presence, but not in the absence of, NADP+ (this cofactor is enzymatically reduced to NADPH by the dehydrogenase present in the reaction mixtures). This result indicates that an oxidative step is involved in the activation of the prodrug. The rate of activation of prodrugs to $\text{MPO}_2\text{-(NHR}^{6-})$ is linearly dependent on microsomal protein concentration, confirming that activation occurs by an enzyme-dependent mechanism.

Example D: Activation of Phosphoramidate Prodrugs by Human Microsomes and the Identification and Tissue Distribution of the Microsomal Enzymes Involved in Activation

Methods: Human liver microsomes and cloned human P450 enzymes representing all major isoforms are obtained from In Vitro Technologies and Gentest Inc., respectively. Reaction mixtures typically contain 100 mM potassium phosphate buffer, pH 7.4, 2 mM NADPH, 0- 2 mg/ml microsomal protein or cloned microsomal isozyme, and the prodrug at, for example, a 100 μM concentration. Formation of $\text{MPO}_2\text{-(NHR}^{6-})$ is monitored by standard reverse phase, ion pairing, or anion exchange HPLC methods. $\text{MPO}_2\text{-(NHR}^{6-})$ is detected by uv absorbance and quantified relative to authentic standards. To determine the tissue distribution of the activating enzyme, homogenates are prepared from key tissues including the liver, lung, intestine, kidney, heart, and skeletal muscle. The rate of conversion of prodrug to $\text{MPO}_2\text{-(NHR}^{6-})$ in each homogenate is assessed as described for the microsomal reactions above. Alternatively, once the major catalytic isoform is identified, immunohistological methods using antibodies raised against the specific enzyme (Waziers et al 1989, J. Pharm. Exp. Ther. 254: 387), or molecular biological methods using PCR techniques (Sumida et al 2000, BBRC 267: 756) are employed to determine the tissue distribution of the activity.

Results: Prodrugs are transformed to $\text{MPO}_2\text{-(NHR}^6\text{)}$ in the human microme-catalyzed reaction at a readily measurable rate. In the screen of cloned microsomal isozymes, the CYP3A4 isozyme catalyzes activation of the prodrugs at the highest rate although other isozymes also show some activity towards the prodrugs. The tissue
5 distribution studies indicate a high activity or abundance of the activating enzyme in the liver relative to other tissues.

Example E: Identification of the P450 Isozyme Involved in Phosphoramidate

Prodrug Activation with use of Specific Enzyme inhibitors

10 Prodrugs are evaluated for human microsome-catalyzed conversion to $\text{MPO}_2\text{-(NHR}^6\text{)}$ in the absence and presence of specific inhibitors of three major P450 isozymes: ketoconazole (CYP3A4), furafylline (CYP1A2), and sulfaphenazole CYP2C9).

Methods: Reactions (0.5 ml @ 37°C) consist of 0.2 M KH_2PO_4 , 13 mM glucose-6-phosphate, 2.2 mM NADP+, 1 unit of glucose-6-phosphate dehydrogenase, 0-
15 2.5 mg/ml human microsomal protein (In Vitro Technologies, Inc.), 250 μM prodrug, and 0-100 μM P450 isozyme inhibitor. Reactions are stopped by addition of methanol to a concentration of 60%, and filtered (0.2 μM filter). $\text{MPO}_2\text{-(NHR}^6\text{)}$ is quantified by standard reverse phase, ion-pairing, or ion exchange HPLC methods. Eluted peaks are quantified relative to authentic standards of known concentration.

20 Results: Ketoconazole inhibits the formation of $\text{MPO}_2\text{-(NHR}^6\text{)}$ in a dose-dependent fashion. The other inhibitors, furafylline and sulfaphenazole, show no significant inhibition. The results indicate that CYP3A4 is the primary P450 isoform responsible for activation phosphoramidate prodrugs in human liver.

25 Example F : Activation of Compound 1.3 by Recombinant CYP3A4

Activation of 1.3 was evaluated in reactions containing microsomes from baculovirus-infected insect cells co-expressing human recombinant CYP3A4 and cytochrome p450 reductase (Panvera Corp., Madison, WI).

Methods: Reaction mixture composition was similar to that described in

30 Example D. Reactions were terminated by addition of methanol to a final concentration of 60% and products were analyzed by HPLC as described in Example A.

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Results: 2-Amino-5-isobutyl-4-[2-(5-phosphonomonoamide)furanyl]thiazole was generated from 1.3 at a rate of 4.2 nmoles/min./nmole CYP3A4.

Example G: Activation of Antiviral Phosphoramidate Prodrugs in Isolated Rat

5 Hepatocytes

The activation of 3.1 and 3.2 was evaluated by monitoring the intracellular generation of the corresponding nucleoside triphosphates in rat hepatocytes.

Methods: Hepatocytes were prepared from fed, dexamethasone-induced Sprague-Dawley rats (300 g) according to the procedure of Berry and Friend (Berry, M.N.,
10 Friend, D.S. J. Cell Biol. 43, 506-520 (1969)) as modified by Groen (Groen, A.K. et al. Eur J. Biochem 122, 87-93 (1982)). Hepatocytes (62 mg wet weight/ml) were incubated in 2 ml Krebs bicarbonate buffer containing 10 mM glucose, and 1 mg/ml BSA. Incubations were carried out in a 95% oxygen, 5% carbon dioxide atmosphere in closed, 50-ml Falcon tubes submerged in a rapidly shaking water bath (37 °C). 3.1 and 3.2 were
15 dissolved in methanol to yield 25 mM stock solutions, and then diluted into the cell suspension to yield a final concentration of 250 µM. Two hours following administration, aliquots of the cell suspension were removed and spun through a silicon/mineral oil layer into 10% perchloric acid. The cell extracts in the acid layers were neutralized by the addition of 0.3 volumes of 3M KOH/ 3M KHCO₃, and the extract was then centrifuged,
20 filtered (0.2 micron) and loaded onto an anion exchange HPLC column equilibrated with 70% A (10 mM ammonium phosphate pH 3.5, 6% ETOH) and 30% B (1 M ammonium phosphate pH 3.5, 6% ETOH). AraATP and acyclovir-triphosphate, the expected metabolites of 3.1 and 3.2, respectively, were eluted from the column with a linear gradient to 80% B in 20 minutes. Detection was by uv absorbance at 254 nm. Peak areas
25 were quantified by comparison of peak areas to authentic standards spiked into naive hepatocyte extracts.

Results: Both prodrugs generated their respective triphosphates in isolated rat hepatocytes. Levels formed following two hours of incubation are summarized below:

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	Compound	Intracellular concentration nmoles/g
5	3.1	15.7±16
	3.2	13.3±3

10

Example H: Kinase Bypass

The generation of acyclovir triphosphate from a phosphoramidate prodrug of acyclovir (Compound **3.2**) and acyclovir itself was compared in rat hepatocytes.

Methods: **3.2** and acyclovir were evaluated in isolated rat hepatocytes at 250
15 μ M as described in Example G.

Results: Levels of acyclovir triphosphate formed following two hours of incubation are summarized below:

20	Compound	Intracellular concentration nmoles/g
	acyclovir	<2
25	3.2	13.3±3

The results demonstrate that a phosphoramidate prodrug of a parent nucleoside such as acyclovir, that is poorly phosphorylated in cells, can bypass the nucleoside phosphorylation step and thus generate higher intracellular levels of triphosphate than the
30 free nucleoside.

Example I: Inhibition of Glucose Production in Rat Hepatocytes by Compound 1.3

Compound **1.3** and its phosphonic acid parent compound, 2-Amino-5-isobutyl-4-(2-furanyl)thiazole, were tested in isolated rat hepatocytes for inhibition of glucose
35 production.

Methods: Isolated rat hepatocytes were prepared from fasted Sprague Dawley rats (250-300 g) as described in Example G. Hepatocytes (75 mg wet weight/ml) were incubated in 1 ml Krebs bicarbonate buffer containing 10 mM glucose, and 1 mg/ml BSA. Incubations were carried out in a 95% oxygen, 5%

carbon dioxide atmosphere in closed, 50-ml Falcon tubes submerged in a rapidly shaking water bath (37 °C). After 10 minutes of equilibration, lactate and pyruvate were added to 10 mM and 1 mM concentrations, respectively. Addition of these gluconeogenic substrates was immediately followed by the addition of test compound to concentrations ranging from 1-100 µM. After 1 hour, an aliquot (0.25 ml) was removed, and transferred to an Eppendorf tube and centrifuged. The resulting supernatant (50 µl) was then assayed for glucose content using a Glucose Oxidase kit (Sigma Chemical Co.) as per the manufacturer's instructions.

Results: **1.3** and 2-Amino-5-isobutyl-4-(2-furanyl)thiazole inhibited glucose production in a dose dependent manner with IC₅₀'s of 8.5 and 2.5 µM, respectively. Since **1.3** is not an inhibitor of FBPase in vitro, inhibition of gluconeogenesis in hepatocytes confirms that prodrug was converted to 2-Amino-5-isobutyl-4-(2-furanyl)thiazole intracellularly. The ~3-fold lower potency of **1.3** relative to 2-Amino-5-isobutyl-4-(2-furanyl)thiazole is consistent with time-dependent activation of prodrug to parent compound in cells.

Example J: Activation of Antidiabetic and Antiviral Phosphoramidate Prodrugs in Induced Rat Hepatocytes

Compounds **1.7** and **12.1** was tested for activation in hepatocytes isolated from rats in which CYP3A4 expression was induced by treatment with dexamethasone. This treatment results in more extensive intracellular activation of phosphoramidate prodrugs.

Methods: Rats were treated with dexamethasone (50 mg/kg, intraperitoneally) for 4 days as described (Brain EGC et al 1998, Br. J. Cancer 7: 1768). Isolated hepatocytes were prepared, and prodrugs tested for activation as described in Example G.

Results: **1.7** activated to yield 20.5±3 nmole/g levels of 2-Amino-5-methylthio-4-[2-(5 phosphono)furanyl]thiazole following two hours of incubation. **12.1** generated 110 ± 0.2 nmoles 1 g acyclovir triphosphate.

Example K: Cytotoxicity Testing of Phosphoramidate Prodrugs in Non-CYP3A4 Expressing Cell Lines

Administration of a drug in a plasma-stable prodrug form can reduce systemic toxicities by limiting exposure to free MPO₂-(NHR⁶⁻) and metabolites. This potential advantage of prodrug delivery is demonstrated by comparing the toxicity profile of the

Methods: The choice of cell line is dictated by the known toxicity profile

of the parent compound. If the toxicity profile of a parent compound is unknown, a panel of different cultured cell lines can be tested. Cells are exposed to a range of prodrug and parent compound concentrations for hours to days. Viability is the measured by Trypan blue exclusion, enzyme marker leakage, incorporation of labeled thymidine into DNA or other standard method.

Results: Phosphoramidate prodrugs are either noncytotoxic or cytotoxic at

considerably higher concentrations than the corresponding parent compound. The latter profile suggests that even if systemic exposure to the prodrug is high, it is likely to show reduced peripheral toxicity in vivo relative to free parent compound. Testing prodrugs in this manner allows an assessment of the intrinsic toxicity of the prodrug and can be used to select prodrugs with favorable cellular safety profiles.

Example L: Cytotoxicity Testing in Cellular Systems that Catalyze Phosphoramidate Prodrug Activation

Cytotoxicity testing in systems in which phosphoramidate prodrugs are activated allows an assessment of the safety of $\text{MPO}_2\text{-(NHR}^6\text{)}$ and other metabolites of the prodrug or parent compound that are generated. Several cellular systems are useful for testing: (a) fresh primary cultures of hepatocytes of human or other origin in which CYP3A4 expression is maintained with an appropriate inducer such as Rifampicin, (b) cell lines in to which CYP3A4 and the requisite reductase are cotransfected, (c) cell lines that are coincubated with primary hepatocytes that express CYP3A4, (d) cell lines that are coincubated with CYP3A4 containing micromal fractions or a cloned CYP3A4 preparation in addition to a standard NADPH recycling system. Prodrugs are incubated in these systems for hours or days. Cytotoxicity is assessed by standard methods such as the Trypan blue exclusion technique, the leakage of enzyme markers, the incorporation of labeled precursors into cellular DNA, etc.

Example M: Byproduct toxicity testing, In Vitro

Activation of certain phosphoramidate prodrugs, in addition to generating $\text{MPO}_2\text{-(NHR}^6\text{)}$, also results in the formation of an electrophilic byproduct capable of alkylating cellular constituents. As the prodrugs in question are activated primarily in the liver,
5 primary rat hepatocytes, known to contain both the microsomal enzymes required for prodrug activation as well as critical detoxification mechanisms, are the cell type of choice for testing the potential toxicity of the byproduct formed.

Methods: Isolated rat hepatocytes are prepared and incubated with test compound as described in Example G. Acetaminophen (0-10 mM) is used as a positive control in
10 these tests since its microsomal metabolism is also known to generate an electrophilic metabolite in these cells (Smolarek TA et al 1989, Metabolism and Disposition 18: 659). This metabolite is detoxified by addition to glutathione, a protective thiol present in mM concentrations in liver cells. Incubations with acetaminophen and phosphoramidate prodrugs are for 4 hours. Cellular viability is assessed by lactate dehydrogenase or
15 other liver enzyme leakage and/or by Trypan blue exclusion. Intracellular glutathione stores are also quantified as described (Baker MA et al 1990, Anal. Biochem. 190: 360).

Results: Acetaminophen depleted intracellular glutathione levels by 90% at the highest dose tested (10 mM) but did not compromise cellular viability as measured by Trypan blue exclusion. The data suggest that the byproduct generated as a result of
20 acetaminophen metabolism was readily detoxified in the hepatocytes, and that the cellular capacity for detoxification was not exceeded. Activation of phosphoramidate prodrugs does not cause byproduct-related toxicities in this hepatocyte system.

Example N: Byproduct toxicity testing, In Vivo

25 Toxicity of the electrophile generated following microsomal activation of certain phosphoramidate prodrugs is tested in the mouse.

Methods: Mice are administered various doses of prodrug and acetaminophen (a positive control, see Example M) and serially sacrificed at various time points following drug administration. Livers are removed and processed for glutathione analysis as
30 described in Example M, and for drug analysis by standard reverse phase, ion-pairing, or ion exchange HPLC methods. Plasma samples are subjected to a standard chemistry panel in which markers of liver function are evaluated.

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Results: Acetaminophen exposure (50, 250 and 500 mg/kg) resulted in a dose-dependent reduction of hepatic glutathione. Liver toxicity in the form of liver enzyme elevations and the appearance of necrotic lesions was evident only at the highest dose, which depleted hepatic glutathione by >80%. This is in accordance with the literature; glutathione forms an adduct with the electrophilic acetaminophen metabolite formed, and protects against liver injury until a threshold level of depletion is reached (Mitchell JR et al 1973, J. Pharm. and Exp. Ther 187: 211). Phosphoramidate prodrug administration also results in glutathione depletion, indicating that the byproduct formed is efficiently detoxified. Liver injury is apparent only at very high doses well above the projected therapeutic doses in man. The data also show dose-dependent accumulation of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites following phosphoramidate prodrug administration.

Example O: Enhanced Delivery of Drugs to the Liver in Animals Pretreated with CYP3A-inducing Agents

Hepatic CYP3A enzymes are induced in rats by treatment with dexamethasone or other suitable agent. Enhanced expression of CYP3A will result in a higher rate of prodrug activation and thus enhance the distribution of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites to this organ.

Methods: Rats are treated with dexamethasone (50 mg/kg, intraperitoneally) for 4 days as described (Brain EGC et al 1998, Br. J. Cancer 7: 1768). Other CYP-inducing agents such as phenobarbital or rifampicin may also be used. The induced animals are then administered prodrug orally or systemically and serially sacrificed at various time points. Livers are removed and homogenized in perchloric acid (10%). Following clarification by centrifugation and neutralization, $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites in the homogenates are quantified by standard HPLC methods. A similar study is conducted in uninduced animals.

Results: The AUC of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites in livers of induced rats will exceed that of uninduced rats. Enhanced liver delivery of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites resulting from CYP3A induction is expected to result in increased efficacy.

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Example P: Oral Bioavailability of Phosphoramidate Prodrugs

The oral bioavailability (OBAV) of phosphoramidate prodrugs is estimated by comparison of the area under the curve (AUC) of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites generated in liver following oral administration to that generated following intravenous administration. Alternatively, OBAV is determined by means of a urinary excretion assay of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites following prodrug administration.

Methods: Rats are dosed orally and systemically with a phosphoramidate prodrug in a suitable vehicle and serially sacrificed at appropriate time point following drug administration. Liver and plasma samples are obtained, processed, and analyzed for $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites by standard reverse phase, ion-pairing, or ion exchange HPLC methods. Alternatively, animals are placed in metabolic cages following drug administration, and urine is collected for 24-48 hours. The quantity of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites excreted into urine is then determined by HPLC analysis. Oral bioavailability is calculated either by comparing the AUC of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites in the liver or the quantity in urine following oral and systemic administration.

Results: The data indicate that phosphoramidate prodrugs are suitable for the oral delivery of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites.

Example Q: Enhanced Liver Delivery and Reduced Systemic Exposure of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its Metabolites Following Administration of Phosphoramidate Prodrugs

Method: Rats are dosed either orally or systemically with a phosphoramidate prodrug, $\text{MPO}_2\text{ (NHR}^{6-})$, and/or its metabolites. Animals are serially sacrificed at appropriate time points following drug administration. Liver and plasma samples are obtained, processed, and analyzed for $\text{MPO}_2\text{ (NHR}^{6-})$ and/or its metabolites by standard reverse phase, ion-pairing, or ion exchange HPLC.

Results: Administration of phosphoramidate prodrugs results in the generation of higher levels of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites in liver and their reduced concentrations in plasma than when free $\text{MPO}_2\text{-(NHR}^{6-})$ is administered.

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Methods: Phosphoramidate prodrugs and respective $\text{MPO}_2\text{-(NHR}^6\text{)}$ are administered systemically to rats. Rats are subsequently caged in metabolic cages and urine collected over a 24-48 hour period. $\text{MPO}_2\text{-(NHR}^6\text{)}$ and/or its metabolites are quantified in urine by means of standard reverse phase, ion-pairing, or ion exchange HPLC methods. By relating the amount of parent compound/metabolites excreted in urine to the dose administered, the % renal clearance is calculated.

Results: Renal excretion of $\text{MPO}_2\text{-(NHR}^6\text{)}$ and/or its metabolites following phosphoramidate prodrug administration is lower than when parent compound is administered. This result indicates that the renal exposure and thus the renal toxicity associated with certain parent compounds and/or their metabolites may be avoided by administration of the compound in phosphoramidate prodrug form.

Methods: Rats are dosed orally and systemically with a phosphoramidate prodrug or the corresponding $\text{MPO}_2\text{-(NHR}^6\text{)}$ and serially sacrificed at appropriate time point following drug administration. Liver samples are obtained, processed, and analyzed for $\text{MPO}_2\text{-(NHR}^6\text{)}$ and/or its metabolites by standard reverse phase, ion-pairing, or ion exchange HPLC. Liver half-lives are determined with the aid of WinNonLin 1.1 software (Scientific Consulting, Inc.).

Results: The half-life of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites in liver is longer when administered in phosphoramidate prodrug form than when free $\text{MPO}_2\text{-(NHR}^{6-})$ is administered. This result is consistent with the prodrug serving as a $\text{MPO}_2\text{-(NHR}^{6-})$ reservoir and, by virtue of a prolonged in vivo half-life, providing sustained release of $\text{MPO}_2\text{-(NHR}^{6-})$ and/or its metabolites to the liver.